Application of non-thermal technologies to enhance the concentration and bioaccessibility of carotenoids in tomato and tomato-based products

Sandra González Casado

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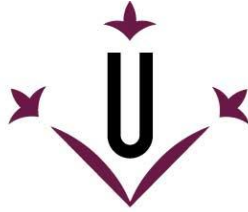

Application of non-thermal technologies to enhance the concentration and bioaccessibility of carotenoids in tomato and tomato-based products

Sandra González Casado

DISSERTATION

to obtain the degree of Doctor by the University of Lleida in the Agricultural and Food Science and Technology program

Directors
Robert Soliva Fortuny
Pedro Elez Martínez

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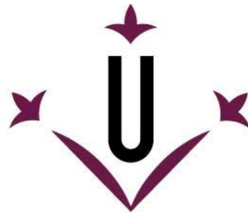

The current research has been performed in the Laboratory of the research unit of Novel Technologies for Food Processing and the Pilot Plant of the Department of Food Technology of the University of Lleida, Spain, under the supervision of Dr. Robert Soliva Fortuny and Dr. Pedro Elez Martínez.

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«En la vida no hay nada que temer y todo que aprender.»

Marie Curie

Dedicado a:

MIS PADRES,

por su incondicional apoyo en todos mis pasos.

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RESUM

La demanda de productes saludables i d'alta qualitat per part dels consumidors ha generat un creixent interès en el desenvolupament de noves estratègies de processament d'aliments que permetin millorar les propietats relacionades amb la salut dels productes alimentaris. En aquest sentit, l'aplicació de polsos elèctrics (PEF) i de llum polsada (PL) està sent investigada com una estratègia innovadora per afavorir l'acumulació de compostos relacionats amb la salut en fruites i hortalisses. L'objectiu de la present Tesi Doctoral va ser avaluar l'efecte de l'aplicació de tractaments de PEF i PL sobre la concentració de carotenoides, així com sobre els principals atributs de qualitat del tomàquet. A més, es van avaluar diversos factors que afecten la concentració i la fracció bioaccesible de carotenoides en els productes derivats de tomàquet.

D'una banda, l'aplicació de tractaments de PEF a fruits de tomàquet va produir un augment significatiu en la concentració de carotenoides totals i licopè. Els tractaments amb una energia de 2.31 $kJ\cdot kg^{-1}$ (2 $kV\cdot cm^{-1}$ i 30 polsos) van produir el major increment de carotenoides totals (50%) i licopè (53%) un cop transcorregudes 24 h des del tractament. Les concentracions dels carotenoides individuals varen variar de manera diferent depenent de les condicions d'emmagatzematge i del tractament de PEF aplicat. En general, els tomàquets emmagatzemats a 12 ºC després del tractament van presentar majors concentracions de carotenoides individuals que els emmagatzemats a 4 o 20 ºC. A més, la intensitat dels tractaments de PEF va condicionar de manera diferent l'acumulació dels carotenoides individuals en els fruits de tomàquet durant l'emmagatzematge. Concretament, l'acumulació de carotenoides individuals en els tomàquets tractats amb una dosi d'energia de 0,38 $kJ\cdot kg^{-1}$ fou més ràpida que en els sotmesos a tractaments més suaus (0,02 $kJ\cdot kg^{-1}$). En concomitància amb l'acumulació de carotenoides, es va produir un augment en l'activitat respiratòria i els canvis en les principals propietats fisicoquímiques dels fruits.

D'altra banda, l'aplicació de tractaments PL de diferent rang espectral va accelerar l'acumulació de carotenoides totals i licopè en els fruits. Els tomàquets sotmesos a tractaments de PL amb longituds d'ona entre 400 i 1100 nm (VIS + NIR) van experimentar el major augment en les concentracions de carotenoides totals (31%) i licopè (35%) durant l'emmagatzematge a 12 ºC sense comprometre els principals atributs de qualitat dels fruits.

El contingut de carotenoides en els derivats del tomàquet es va veure significativament afectat per l'estat de maduresa dels fruits, el tipus de processament mecànic, l'addició i tipus d'oli i l'aplicació de tractaments de PEF als fruits abans del processat. Les majors concentracions de carotenoides es van trobar en els derivats de tomàquet obtinguts a partir de fruits completament madurs. D'altra banda, l'alteració de la integritat tissular mitjançant triturat va comportar una notable disminució del contingut de carotenoides totals i licopè, mentre que l'addició d'oli va jugar un paper protector enfront de la degradació de carotenoides com a conseqüència del processat mecànic. Finalment, l'aplicació de PEF a fruits sencers augmentar significativament la concentració de carotenoides individuals en els derivats obtinguts a partir d'aquests. En general, la concentració màxima de compostos carotenoides es va trobar en els purés de tomàquet obtinguts a partir de fruits tractats amb 2 $kV \cdot cm^{-1}$ i 30 polsos (2.31 $kJ \cdot kg^{-1}$).

Els factors que van condicionar la concentració de carotenoides en els productes transformats també van afectar la seva bioaccesibilitat. En aquest sentit, a mesura que el tomàquet madurava, la fracció bioaccessible de carotenoides totals i licopè en els productes derivats es va veure incrementada. A més, l'alteració de la matriu va facilitar l'alliberament de carotenoides i la seva micel·larització, donant com a majors bioaccesibilitats. A més, els productes de tomàquet amb un 5% d'oli d'oliva afegit van presentar els majors valors de bioaccesibilidad de carotenoides, seguits per aquells en els quals es va afegir oli de gira-sol i coco. Finalment, l'aplicació de tractaments de PEF als fruits de tomàquet va augmentar significativament la fracció bioaccesible dels carotenoides individuals en els purés obtinguts amb oli d'oliva afegit.

L'aplicació de tractaments de PEF a 2 kV·cm-1 i 5 polsos (0,38 kJ·kg^{-1}) va produir l'augment màxim de la bioaccesibilitat dels carotenoides totals (37%) així com de la majoria dels carotenoides individuals.

Els resultats obtinguts evidencien el potencial de les tecnologies avaluades per obtenir tomàquets i productes derivats amb alt contingut de carotenoides. En conseqüència, els resultats d'aquesta Tesi Doctoral ofereixen noves perspectives a la indústria transformadora per al desenvolupament de derivats de tomàquet d'alta qualitat i més saludables.

RESUMEN

La demanda de productos saludables y de alta calidad por parte de los consumidores ha generado un creciente interés en el desarrollo de nuevas estrategias de procesado de alimentos que permitan mejorar las propiedades relacionadas con la salud de los productos alimenticios. En este sentido, la aplicación de pulsos eléctricos (PEF) y de luz pulsada (PL) está siendo investigada como una estrategia innovadora para favorecer la acumulación de compuestos bioactivos en frutas y hortalizas. El objetivo de la presente Tesis Doctoral fue evaluar el efecto de la aplicación de tratamientos de PEF y PL sobre la concentración de carotenoides, así como sobre los principales atributos de calidad del tomate. Además, se evaluaron diversos factores que afectan a la concentración y a la fracción bioaccesible de carotenoides en los productos derivados de tomate.

Por un lado, la aplicación de tratamientos de PEF a frutos de tomate produjo un aumento significativo en la concentración de carotenoides totales y licopeno. Los tratamientos con una energía de 2.31 $kJ \cdot kg^{-1}$ (2 $kV \cdot cm^{-1}$ y 30 pulsos) produjeron el mayor incremento de carotenoides totales (50%) y licopeno (53%), transcurridas 24 h desde el tratamiento. Las concentraciones de los carotenoides individuales variaron de manera diferente dependiendo de las condiciones de almacenamiento y del tratamiento de PEF aplicado. En general, los tomates almacenados a 12 ºC después del tratamiento presentaron mayores concentraciones de carotenoides individuales que los almacenados a 4 o 20 ºC. Además, la intensidad de los tratamientos de PEF condicionó la acumulación de los carotenoides individuales en los frutos de tomate durante el almacenamiento. Concretamente, la acumulación de carotenoides individuales en los tomates tratados con una dosis de energía de 0,38 $kJ \cdot kg^{-1}$ fue más rápida que la de los frutos tratados con tratamientos más suaves (0,02 $kJ \cdot kg^{-1}$). En concomitancia con la acumulación de carotenoides, se produjo un aumento de la actividad respiratoria y cambios en las principales propiedades fisicoquímicas de los frutos.

Por otro lado, la aplicación de tratamientos PL de diferente rango espectral aceleró la acumulación de carotenoides totales y licopeno en los frutos. Los tomates sometidos a tratamientos de PL con longitudes de onda entre 400 y 1100 nm (VIS+NIR) experimentaron el mayor aumento en las concentraciones de carotenoides totales (31%) y licopeno (35%) durante el almacenamiento a 12 ºC sin comprometer los principales atributos de calidad de los frutos.

El contenido de carotenoides en los derivados del tomate se vio significativamente afectado por el estado de madurez de los frutos, el tipo de procesamiento mecánico, la adición y el tipo de aceite, así como por la aplicación de tratamientos de PEF a los frutos antes del procesado. Las mayores concentraciones de carotenoides se encontraron en los derivados de tomate obtenidos a partir de frutos completamente maduros. Por otro lado, la alteración de la integridad tisular mediante trituración produjo una notable disminución del contenido de carotenoides totales y licopeno, mientras que la adición de aceite jugó un papel protector frente a la degradación de carotenoides a causa del procesado mecánico. Finalmente, la aplicación de PEF a frutos de tomate aumentó significativamente la concentración de carotenoides individuales en los derivados obtenidos a partir de éstos. En general, la concentración máxima de compuestos carotenoides se encontró en los purés de tomate obtenidos a partir de frutos tratados con 2 $kV \cdot cm^{-1}$ y 30 pulsos (2.31 $kJ \cdot kg^{-1}$).

Los factores que condicionaron la concentración de carotenoides en los productos transformados también afectaron a su bioaccesibilidad. En este sentido, a medida que el tomate maduraba, la fracción bioaccessible de carotenoides totales y licopeno en los productos derivados se vio incrementada. Además, el triturado facilitó la liberación de carotenoides y su micelarización, dando como resultado mayores bioaccesibilidades. Además, los productos de tomate con un 5% de aceite de oliva añadido presentaron los mayores valores de bioaccesibilidad de carotenoides, seguidos por aquellos a los que se añadió aceite de girasol y coco. Por último, la aplicación de tratamientos de PEF a los frutos de tomate aumentó significativamente

la fracción bioaccesible de los carotenoides individuales en los purés con aceite de oliva añadido. La aplicación de tratamientos de PEF a 2 $kV \cdot cm^{-1}$ y 5 pulsos (0,38 $kJ \cdot kg^{-1}$) produjo el aumento máximo de la bioaccesibilidad de los carotenoides totales (37%) así como de la mayoría de los carotenoides individuales.

Los resultados obtenidos evidencian el potencial de las tecnologías evaluadas para obtener tomates y productos derivados con alto contenido en carotenoides. Por lo tanto, los resultados de esta Tesis Doctoral ofrecen nuevas perspectivas a la industria transformadora para el desarrollo de nuevos productos de alta calidad y más saludables.

ASTRACT

The consumers' demand of high quality and healthy products has triggered a growing interest on the development of new processing strategies that allow enhancing the health-related properties of food products. In this sense, the application of pulsed electric fields (PEF) and pulsed light (PL) treatments is investigated as an innovative strategy to elicit the stress-mediated accumulation of health-related compounds in fruits. The objective of the present Doctoral Thesis was to evaluate the effect of PEF and PL treatments on the carotenoid concentration as well as on the main quality attributes of tomato fruits. In addition, the factors affecting the concentration and bioaccessible fraction of carotenoids in tomato-based products were also evaluated.

On the one hand, the application of PEF treatments to intact tomato fruits resulted in a significant increase in the concentration of both total carotenoid and lycopene. Treatments of a delivered energy of 2.31 $kJ \cdot kg^{-1}$ (2 $kV \cdot cm^{-1}$ and 30 pulses) led to the greatest enhancements of total carotenoid (50%) and lycopene (53%) contents within the following 24 h period. The concentration of each individual carotenoid was differently affected by the storage conditions depending on the previously applied PEF treatment. In general, tomatoes stored at 12 ºC after processing exhibited higher individual carotenoid concentrations than those stored at 4 or 20 ºC. Moreover, the intensity of the PEF treatments differently affected the accumulation of individual carotenoids in tomato fruits throughout storage. Thus, the accumulation of individual carotenoids in tomatoes treated with an energy dose of 0.38 $kJ \cdot kg^{-1}$ was faster than those found in fruits treated with milder PEF treatments (0.02 $kJ \cdot kg^{-1}$). In concomitance with the accumulation of carotenoids, an increase in the respiratory activity and changes in the main physicochemical properties of the fruits were observed.

On the other hand, the application of PL treatments of different spectral range accelerated the accumulation of total carotenoids and lycopene in tomato fruits. Tomatoes subjected to PL treatments

delivering wavelengths ranging from 400 to 1100 nm (VIS+NIR) exhibited the greatest enhancements in total carotenoid (31%) and lycopene (35%) concentrations throughout 5 days of storage at 12 ºC without compromising the main quality attributes of the fruits.

The content of carotenoids in tomato derivatives was shown to be significantly affected by the ripeness stage of the fruits, the type of mechanical processing, the addition of oil and the application of PEF treatments to the intact tomato fruits before processing. The highest carotenoid concentrations were found in tomato-based products obtained from fully-ripe fruits. The disruption of tomato tissues by grinding led to a remarkable decrease in total carotenoid and lycopene contents, whereas the addition of oil played a protective role against carotenoid degradation during mechanical processing. Eventually, the application of PEF to whole fruits as a pre-processing treatment significantly increased the concentration of individual carotenoids in the subsequently obtained added-olive oil tomato derivatives. In general the maximum concentration of carotenoid compounds was found in tomato purees obtained from fruits treated with 2 $kV \cdot cm^{-1}$ and 30 pulses (2.31 $kJ \cdot kg^{-1}$).

The factors affecting the carotenoid concentrations in tomato products also influenced their bioaccessibility. In this regard, tomato fruit ripening significantly increased the bioaccessible fraction of total carotenoids and lycopene in the derived products. The disruption of the food matrix facilitated the release of carotenoids and their micellarization, thus resulting as well in greater bioaccessibility values. In addition, tomato products with 5% of added olive oil exhibited the greatest values of carotenoid bioaccessibility, followed by those obtained by adding sunflower and coconut oils. Finally, the application of PEF treatments to tomato fruits significantly enhanced the bioaccessible fraction of individual carotenoids in the obtained oil-added purees. PEF treatments conducted at 2 $kV \cdot cm^{-1}$ and 5 pulses (0.38 $kJ \cdot kg^{-1}$) exhibited the maximum increase in total carotenoids bioaccessibility (37%) as well as in most individual carotenoids.

The results obtained evidence the potential of the evaluated technologies to obtain tomatoes and tomato derivatives with high content of carotenoids and enhanced health-related properties. Therefore, the results of this Doctoral Thesis offer new prospects to industrial processors for developing healthier high-quality tomato derivatives.

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INTRODUCTION

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1. Tomato and tomato products

Tomato is one of the most important vegetable crops worldwide. It is grown in practically every country of the world in outdoor fields, greenhouses, home gardens or even in potted plants. The world annual production of tomato has continuously increased during the last decade (Figure 1), reaching about 170 million tons in 2014 (FAO 2016). This fact makes tomato the eleventh agricultural commodity with higher world annual production (Martínez-Hernández et al. 2015).

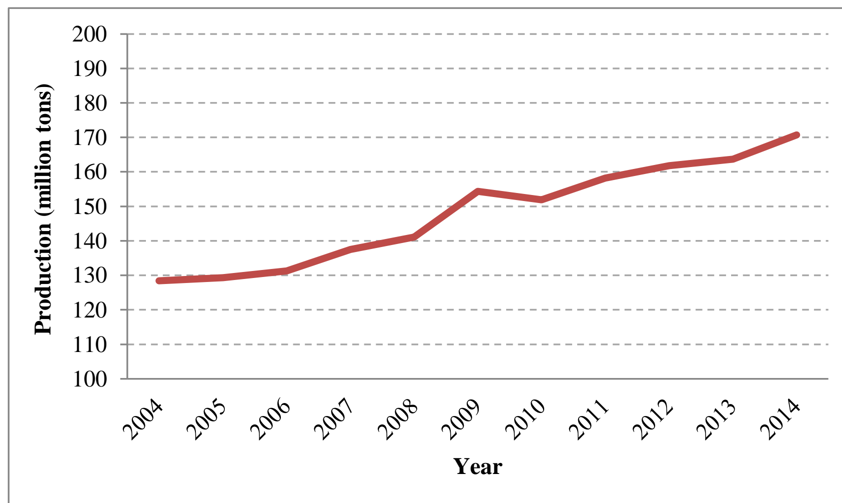

Figure 1. *World tomato production (million tons) (Created from FAO, 2016).*

China is the largest tomato producer in the world, followed by the United States, Turkey, India, Egypt and Italy (MAPAMA, 2016). These countries account for 70% of global production. According to Eurostat (2011), Spain cultivates 28% of the tomatoes produced in Europe, making the country the second largest producer in the European Union (EU) behind Italy.

Tomato production is normally destined to the fresh market or the processing industry. Because of its versatility, there is a large list of different tomato-based products, such as ketchup, tomato paste, whole

peeled tomatoes, chilli sauce, pizza and pasta sauces, juice and "gazpacho soup", among others.

Annual consumption of tomatoes, either fresh or processed, in EU was 33 kg per capita (Eurostat, 2011). Because of this high consumption, tomato has become an important component in the European diet. In particular, it is a key component in the so-called "Mediterranean diet", which is strongly associated with a healthy lifestyle (Martínez-Hernández et al. 2015; Vallverdú-Queralt et al. 2011).

2. Carotenoids of tomato

2.1. Carotenoids as bioactive compounds

Bioactive compounds are considered extranutritional constituents that normally occur in small quantities in foods (Kris-Etherton et al. 2002). Nonetheless, researchers have focused on these food components because of the growing evidence about the potential health benefits of their consumption. Fruits and vegetables are considered as the main dietary sources of bioactive compounds. Many of these phytochemicals are antioxidants, which can inactivate certain harmful free radicals in the body (Hedges & Lister 2005). The predominant bioactive compounds found in fruits and vegetables are carotenoids, flavonoids, glucosinates, lignans, monoterpenes, organsulfur compounds, phenolic acids, phytosterols, saponins, stilbenes and tannins (Kris-Etherton et al. 2004).

Carotenoids are a family of isoprene-derived lipophilic pigments that naturally have 40-carbon molecules and multiple conjugated double bonds (Failla, Huo, et al. 2008). These compounds are typically found in higher plants, algae and numerous bacteria and fungi (Hedges & Lister 2005). Animals cannot biosynthesize carotenoids de *novo*, hence their intake from food is needed (Hornero-Méndez & Mínguez-Mosquera 2007). Chemically, carotenoids can be divided into two major classes: (i) xantophylls, which are molecules containing one or more oxygenated group substituents at particular sites on the terminal rings, such as lutein and zeaxanthin, and (ii) carotenes, that are unoxygenated

carotenoids, such as lycopene, β-carotene and α-carotene (Shi & Maguer 2000). More than 700 carotenoids have been isolated, but only around 40-50 play a role in the human diet (Bohn 2008).

With regard to tomato, this fruit contains an array of bioactive compounds, including vitamin C and carotenoids, and also provides relevant amounts of vitamin E, flavonoids, as well as other phenolic compounds (Hedges & Lister 2005). Tomato is considered as one of the most important sources of carotenoids in the human diet (Shi & Maguer 2000). Among these, lycopene, the main responsible compound for its characteristic deep-red colour, is the most abundant carotenoid in ripe tomatoes (Guil-Guerrero & Rebolloso-Fuentes 2009). Tomatoes also contain other minor carotenoids, including phytoene, phytofluene, neurosporene, β-carotene, δ-carotene, γ-carotene, α-carotene and lutein (Fraser et al. 1994; Clinton 1998; Vallverdú-Queralt, Oms-Oliu, et al. 2013). Because of their characteristic structure, rich in conjugated double bonds, carotenoids can appear as *cis*- or *trans*-isomers. *Trans*-configuration is the most common form of carotenoids found in fresh tomatoes (Arranz et al. 2015). The isomerization into *cis*-form could occur during tomato processing and storage (Shi & Maguer 2000). However, the conversion into *cis* isomers affects the biological properties of carotenoids (Boileau et al. 2002).

2.2. Health-related effects of carotenoids

Carotenoids show free radical scavenging properties, either due to direct quenching reactions with singlet oxygen or reaction with radicals such as peroxyl ($ROO^{\cdot}$) or hydroxyl ($OH^{\cdot}$), hence acting as powerful antioxidants (Bohn 2008). The antioxidant activity of carotenoids mainly depends on their chemical structure, especially the number of conjugated double bonds and the nature of end groups (cyclic or acyclic) (Stahl & Sies 1996). In addition, approximately 10% of these carotenoids act as precursors of vitamin A (retinol) (Yeum & Russell 2002; Fernández-García et al. 2012). In humans, this vitamin is required for vision, cell differentiation, and the immune system (Failla, Huo, et al. 2008). The most important ones, as a consequence of their high

provitamin-A activity level, are β-carotene, α-carotene, some xantophylls, and apocarotenoids (Castenmiller & West 1998; Fernández-García et al. 2012). Although lycopene has no provitamin A activity, it shows a constant physical quenching rate with singlet oxygen, which is almost twice as high as that of β-carotene, thus providing an effective protection against a broad range of epithelial cancers and cardiovascular diseases (Shi & Maguer 2000; Hedges & Lister 2005).

2.3. Carotenoid biosynthesis

In plants, carotenoids are synthesized as secondary metabolites besides the primary biosynthetic and metabolism routes to assure the normal growth and development of plants (Paulsen 2010). Carotenoid biosynthesis in higher plants depends on the supply of both the isopentenyl diphosphate (IPP) and its isomer dimethylallyl diphosphate (DMAPP), which are the precursor molecules of all carotenoids (Liu et al. 2015). Two distinct pathways for IPP and DMAPP biosynthesis exist: the cytosolic mevalonic acid pathway (MVA) and the plastidic methylerythritol 4- phosphate (MEP) pathway (DellaPenna & Pogson 2006). Four molecules of IPP are subjected to a series of condensation reactions in order to form geranylgeranyl diphosphate (GGDD), which is a key intermediate in the synthesis of carotenoids (Bohn 2008). The condensation of two molecules of GGDP by phytoene synthase (PSY) to form phytoene is considered to be the first committed step in plant carotenoid biosynthesis (Figure 2). Several studies have reported that PSY is a rate-limiting step which regulates carotenoid metabolic flux (Lu & Li 2008). Phytoene is desaturated by phytoene desaturase (PDS) into phytofluene, and subsequently by ζ-carotene desaturase (ZDS) into red-coloured lycopene. The cyclation of lycopene is the branching point in the carotenoids biosynthetic pathway. One route leads to γ-carotene and β-carotene by lycopene β-cyclase (LCYB). In the alternative pathway, lycopene is cyclized either to form α-carotene by lycopene ε-cyclase (LCYE) and lycopene β-cyclase (LCYB). Then, α-carotene and β-carotene are hydroxylated to produce lutein and zeaxanthin, respectively. Further epoxidation and de-epoxidation reactions may

occur, resulting in different compounds, such as violaxanthin, neoxanthin and capsanthin, among others. The synthesized carotenoid end products can be catabolized to produce apocarotenoids and abscisic acid (ABA) (DellaPenna & Pogson 2006; Bohn 2008; Lu & Li 2008; Liu et al. 2015; Yahia & Ornelas-Paz 2009).

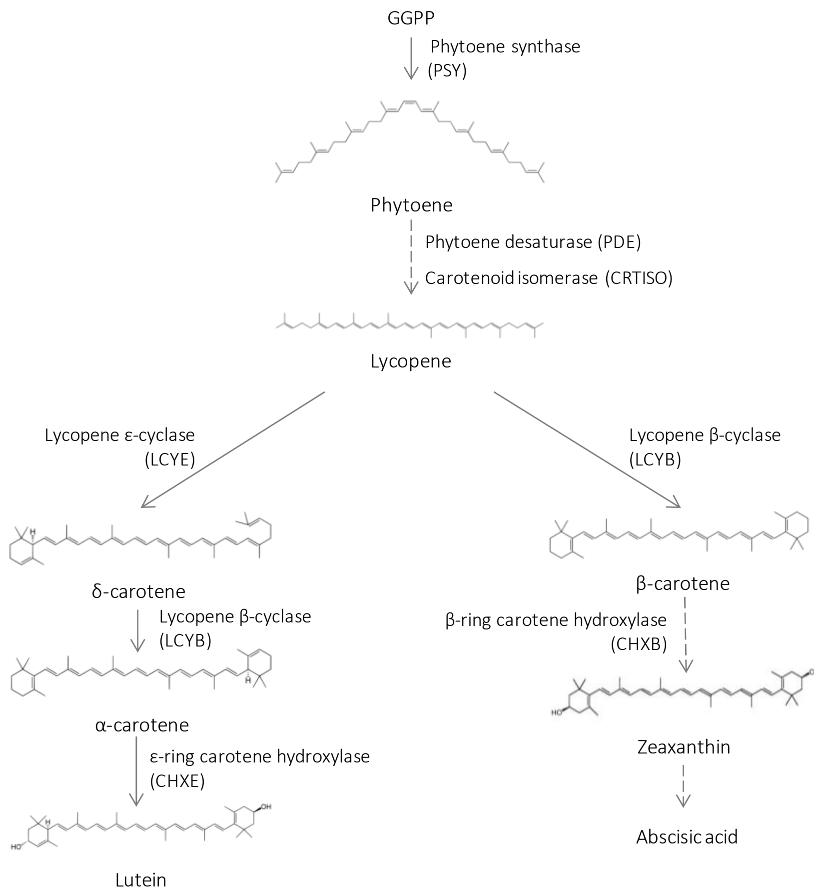

Figure 2. *Carotenoids biosynthetic pathway of tomato (adapted from* DellaPenna & Pogson 2006; Liu et al. 2015).

2.4. Factors affecting the concentration of carotenoids in tomato and tomato-based products

Several factors have been reported to affect the amount of carotenoids present in tomato and tomato derivatives. Changes in the concentration of these compounds can compromise the quality and, eventually, the acceptance of tomato products. Ripeness stage, the imposition of stress signals, postharvest handling, and processing can be pointed out among the most important factors affecting the overall concentration of carotenoids in tomato and tomato derivatives.

2.4.1. Ripeness stage

Tomato fruits are often harvested at different ripeness stages from green-orange (breaking) to red-ripe stage, depending on the consumer and market preference. However, the amount of carotenoids can vary considerably between different ripeness stages. Ripening of tomato is associated to morphological, physiological and biochemical changes including the disappearance of chlorophylls and the accumulation of carotenoids within the plastids (Ilahy et al. 2011). In this regard, several authors have reported a continuous increase in the concentration of the main carotenoids of tomato during fruit development, especially lycopene (Cano et al. 2003; Ilahy et al. 2011). The rapid and large accumulation of carotenes, particularly lycopene and β-carotene, can be due to an enhanced activity of the enzymes that regulate the biosynthetic pathway of carotenoids (Fraser et al. 1994). Moreover, ripening also involves a series of metabolic reactions, finally resulting in dramatic changes in colour, flavour and texture of tomatoes, which affect the final appearance of the fruits. Therefore, nutritional and sensorial characteristics can markedly vary depending on the ripeness stage of tomato.

2.4.2. Stress signals

Diverse environmental factors affect plants in various ways which can be either beneficial or detrimental to their productivity. In plant sciences, the terms of "stress factor" or "stressor" are used to describe an imposed external factor that can produce changes in growth and development patterns (Hideg et al. 2013). There are a number of exogenous stresses which have been recognized to be potentially harmful to plants (Figure 3) (Choudhury et al. 2013; Balasa 2014; Cramer et al. 2011). However, through the history of evolution, plants have developed a wide variety of efficient mechanisms to sense, respond and adapt to these environmental changes (Fraire-Velazquez & Emmanuel 2013). One of the most important is the bioproduction of secondary metabolites. These compounds are usually referred as compounds that have no fundamental role in the maintenance of life processes in plants, but they are important to interact with the environment for adaptation and defence (Ramakrishna & Ravishankar 2011).

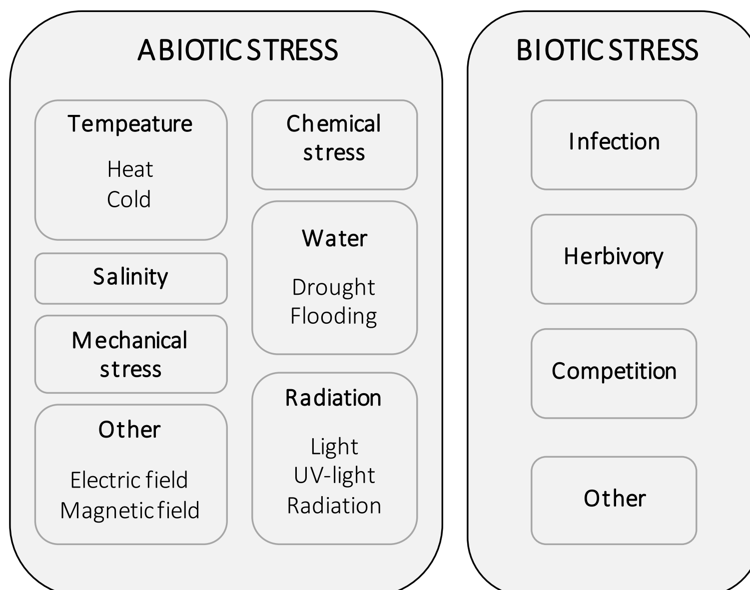

Figure 3. *Abiotic and abiotic external stress factors (adapted from Balasa, 2014 and Ramakrishna & Ravishankar, 2011).*

Environmental abiotic stress often results in a rapid production of reactive oxygen species (ROS), including superoxide (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl (OH^-) radicals, which generate an oxidative imbalance in plant cells (Galindo et al. 2007). The excess of oxidative stress causes severe damage to protein structures, inhibits the activity of multiple enzymes of important metabolic pathways and results in oxidation of macromolecules including lipids and DNA. All these adverse events compromise the cellular integrity and may lead to intoxication and cell death (Fraire-Velazquez & Emmanuel 2013). In order to scavenge the deleterious effect of ROS, plants activate their own antioxidant defense system (Sharma et al. 2012). Thus, several authors have suggested that the generation of ROS may trigger the signal required to activate the metabolic pathway of secondary metabolites, such as polyphenolic compounds and carotenoids, leading to their accumulation in plants (Ramakrishna & Ravishankar 2011; Łukaszuk, E. & Ciereszko 2012; Balasa & Knorr 2011; Vallverdú-Queralt, Oms-Oliu, et al. 2013).

2.4.3. Postharvest handling

The production of bioactive compounds is a complex process, associated to ripening, which is influenced by environmental and genetic factors during plant development (Javanmardi & Kubota 2006). However, biochemical compounds, especially carotenoids, could also be influenced during postharvest storage. Being a climacteric fruit, tomato metabolism continues after the detachment from the plant (Toor & Savage 2006). Primary and secondary metabolites are biosynthesized during postharvest storage concurrently with other processes which lead to the modification of colour, flavour, texture and chemical composition (Balasa 2014). Under optimized storage conditions the biosynthesis of secondary metabolites and volatiles and the degradation of chlorophylls can normally occur, allowing maintaining the optimal fruit quality. However, improper storage conditions could cause some deleterious effects on quality-related compounds (Vinha et al. 2013).

The most important storage condition among those affecting the carotenoids concentration of tomato fruits is temperature. Temperature plays an important role in maintaining the postharvest quality of tomato. The rate of metabolic processes, including the development of red colour, is strongly affected by storage temperature (Vinha et al. 2013; Toor & Savage 2006; Tadesse et al. 2015). Some authors have reported that low storage temperatures (below 12 ºC) deleteriously affect the rate of ripening and flavour development in tomatoes, due to chilling injury associated phenomena (Tadesse et al. 2015; Javanmardi & Kubota 2006). In this case, chlorophyll is not easily degraded and lycopene accumulation does not take place (Vinha et al. 2013). On the contrary, previous studies report an increase in colour development, related to increase lycopene concentrations, when tomatoes are stored at temperatures higher than 12 ºC (Vinha et al. 2013). High storage temperatures impact not only carotenoid concentrations and colour, but also other physicochemical properties such as texture and acidity, which are associated to the acceptability of the fruits (Tadesse et al. 2015; Vinha et al. 2013).

2.4.4. Processing

It is well established that processing of tomato products has an important influence on their carotenoids content. Due to their molecular configuration, rich in conjugated doubled bonds, carotenoids are very sensitive to heat, light, oxygen, and pH. Hence, carotenoids may undergo degradation via isomerization and/or oxidation during tomato processing (Martínez-Hernández et al. 2015).

The analysis of the effect of traditional food processing on the concentration of carotenoids has been widely evaluated. In this sense, several authors have reported that the operations that reduce the particle size of tomato matrix, such as grinding, chopping, milling or homogenization, produce a significant loss of carotenoids (Takeoka et al. 2001; Martínez-Hernández et al. 2015). In addition, thermal treatments are the main cause of the depletion of natural antioxidants

in food, especially under severe processing conditions (Vallverdú-Queralt et al. 2015; Shi & Maguer 2000).

Furthermore, emerging non-thermal technologies, such as pulsed electric fields (PEF) and pulsed light (PL) are currently undergoing extensive research because of their ability to inactivate microorganisms avoiding thermal degradation of the food components, and consequently preserving the sensory and nutritional quality of the processed food products. However, it has been demonstrated that the application of these technologies could offer other useful applications for the food industry. In this regard, PEF treatments of low/moderate intensity have been proposed as a new strategy to enhance the concentration of some nutritionally valuable phytochemicals in fruits and vegetables (Balasa 2014; Vallverdú-Queralt, Oms-Oliu, et al. 2013). PEF can act as an abiotic stressor leading to the activation of the secondary metabolism of plant tissues as a way to overcome unfavourable conditions (Galindo et al. 2009). As a result, an accumulation of health-related compounds, such as carotenoids and phenolic compounds, could occur (Balasa & Knorr 2011; Vallverdú-Queralt et al. 2012). On the other hand, it has been recently reported that the application of postharvest UV-C and pulsed light treatments also enhanced the accumulation of carotenoids in tomato fruits (Pataro et al. 2015; Aguiló-Aguayo et al. 2013). However, there is very little information available concerning the application of either PEF or PL treatments to whole tomatoes as a novel strategy to produce both fruits and tomato-based products with high health-related properties.

2.5. Bioaccessibility of carotenoids

The nutritional value of food products is usually given by the content of food constituents. Nevertheless, the bioaccessible fraction of nutrients is more relevant than the total amount present in the original food (Rodríguez-Roque 2014; Knockaert, Pulissery, et al. 2012). Several researchers have proposed the term bioaccessibility as a previous approach to estimate the nutritional value of food products. Bioaccessibility is defined as the fraction of the ingested food

constituent that is released from the food matrix in the gastrointestinal tract during digestion and thus becomes available for intestinal absorption (Cardoso et al. 2015). During the last years, different *in vitro* methodologies have been proposed to assess the bioaccessibility of bioactive compounds of food products.

2.5.1. *In vitro* gastrointestinal digestion

In vitro gastrointestinal digestion has been proposed as a fast, simple, cheap and reproducible approach for assessing the bioaccessibility of different food constituents (Rodríguez-Roque 2014). This method allows determining the amount of certain nutrient and/or bioactive compound that could be released from the food matrix and become available for intestinal absorption. A number of *in vitro* gastrointestinal digestion methods has been developed during the last decades (Hur et al. 2011). These methods are aimed at simulating the physiological conditions that occur during digestion in the human gastrointestinal tract, taking into account the presence of digestive enzymes and their concentrations, pH, digestion time, and salt concentrations, among other factors (Minekus et al. 2014). Simulated digestion typically includes the oral, gastric and small intestinal phases, and occasionally large intestinal fermentation (Figure 4).

With regard to carotenoids, their uptake follows the same fate as lipids because of their lipophilic behaviour. In this sense, carotenoids must be released from the food matrix during the gastric phase of digestion and solubilised into mixed micelles (Failla, Huo, et al. 2008). The micellarization is a critical step for the carotenoids absorption because only carotenoids incorporated into micelles can be taken up by intestinal cells (Alminger et al. 2012). Therefore, the quantification of the amount of carotenoids transferred from food matrix to the aqueous-micellar phase represents the potential for carotenoids absorption (Van Buggenhout et al. 2010).

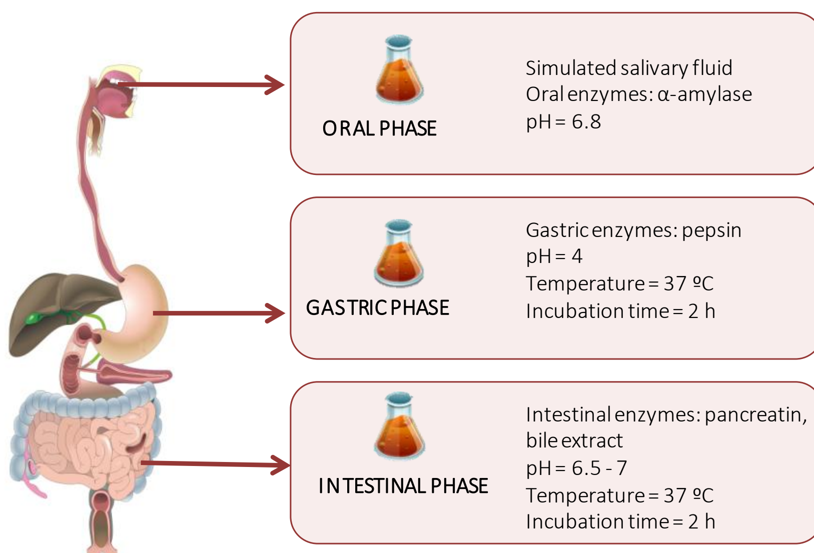

Figure 4. *In vitro gastrointestinal digestion scheme (Schematic representation of the human digestive apparatus retrieved from the database of the Spanish Ministry of Education).*

2.5.2. Factors affecting the bioaccessibility of carotenoids

The bioaccessibility of carotenoids is extremely variable and may be influenced by several factors. Studies suggest that different matrices in plant foods at different ripeness stages may affect carotenoid bioaccessibility (Ornelas-Paz et al. 2008). Ripening implies morphological, physiological, biochemical and molecular changes including chlorophyll degradation and accumulation of carotenoids (Ilahy et al. 2011). Therefore, both nutritional and health-related properties change during fruit development. However, to the best of our knowledge there are no previous studies assessing the influence of ripeness stage of tomato in carotenoids bioaccessibility.

It is important to point out that the *in vitro* bioaccessibility varies widely for different carotenoids. Thus, xanthophylls have been reported to be more efficiently transferred into mixed micelles than carotenes (Granado-Lorencio et al. 2007). Tyssandier, Lyan, & Borel (2001) suggested that the bioaccessibility depends on the hydrophobicity of carotenoids, which is determined by their chemical structure. It is possible that less apolar xanthophylls, which are mainly located in the phospholipid surface of the lipid droplets, are more easily transferred into micelles than more apolar carotenes mainly located in the triacylglycerol core of the droplets (Tyssandier et al. 2001).

As mentioned before, once the lipophilic carotenoids are released from food matrix, they have to be incorporated into micelles. Therefore, the ingestion of fat with carotenoids has been shown to be crucial for the carotenoids absorption. Thus, some studies have demonstrated that carotenoids bioaccessibility is enhanced when lipids are added during processing and/or digestion (Colle et al. 2012; Hedren et al. 2002). Subsequent *in vivo* studies have confirmed that fat is likely the most important dietary promoter of carotenoid absorption (Arranz et al. 2015; Brown et al. 2004). Moreover, other authors have evaluated the influence of the dietary fatty acid characteristics (length and degree of unsaturation) on the bioaccessibility of carotenoids (Huo et al. 2007; Nagao et al. 2013). However, the amount and type of dietary fat needed to achieve the optimal carotenoid bioaccessibility is not clear.

On the other hand, the food matrix has also been demonstrated to play an important role in carotenoids bioaccessibility. It is known that food processing facilitates the disruption of food matrix, thus leading to the liberation of carotenoids and their solubilization into the micellar phase. Hence, the carotenoids bioaccessibility could be enhanced after processing (Rodríguez-Roque et al. 2015; Svelander et al. 2011; Martínez-Hernández et al. 2015; Ryan et al. 2008; Hornero-Méndez & Mínguez-Mosquera 2007; Knockaert, Pulissery, et al. 2012). Moreover, carotenoids may undergo degradation via oxidation and isomerization during processing, which could affect the bioavailability of these compounds (Martínez-Hernández et al. 2015). Bohm et al. (2002)

concluded that the isomerization of carotenoids is desirable because *cis*-isomers have higher antioxidant activity than those in *trans*-form. In addition, *cis*-isomers are more easily taken up by mixed micelles in the intestine, and hence are more bioavailable compared to *trans*-compounds (Boileau et al. 2002). Therefore, it seems that processing of tomato has an important impact on the carotenoids content and its bioaccessibility.

3. Non-thermal technologies

3.1. Pulsed electric fields

3.1.1. Fundamental aspects of PEF processing

Pulsed electric fields (PEF) are considered as a non-thermal technology for food processing. PEF treatments involves the application of a short burst (µs or ms) of high voltage energy to food products (Martín-Belloso & Elez-Martínez 2005). A typical PEF system consists of a voltage source, a capacitor bank and a switch (Altunakar & Barbosa-Cánovas 2011). A key element of PEF systems is the treatment chamber, wherein the food is exposed to the electric field pulses delivered through two stainless steel electrodes (Figure 5).

The generation of pulsed electric fields needs slow charging and fast discharging of the energy (Toepfl et al. 2005). The charging voltage required to pulse generation is highly dependent of the electrode distance. Thus, for two parallel electrodes the electric field strength (E) is given by equation 1:

$$E = \frac{U}{d} \quad (1)$$

where U is the voltage (kV) and d the interelectrode distance (m).

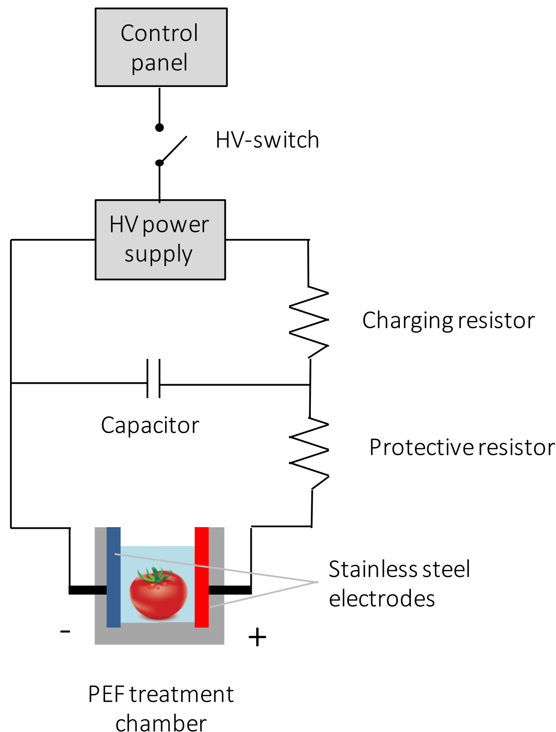

Figure 5. *Simplified circuitry of a pulsed electric fields system (adapted from Martín Belloso & Soliva Fortuny 2011)*

The application of an external electrical field with sufficient strength will induce the accumulation of charges at the cell membranes, resulting in a naturally occurring transmembrane potential. Accumulation of opposite charges on both sides of the membrane raises compression pressure on the cell membrane and induces its thinning (Zimmermann 1986). When the threshold value of this transmembrane potential is exceeded, pore formation will occur (Toepfl et al. 2005) (Figure 6). This phenomenon is commonly called permeabilization or electroporation. Critical electric field strength to induce membrane permeabilization is dependent on the geometry and size of a cell, in the range of 1 - 2 kV/cm for plant cells and in the range of 12-20 kV/cm for microorganisms (Soliva-Fortuny et al. 2009).

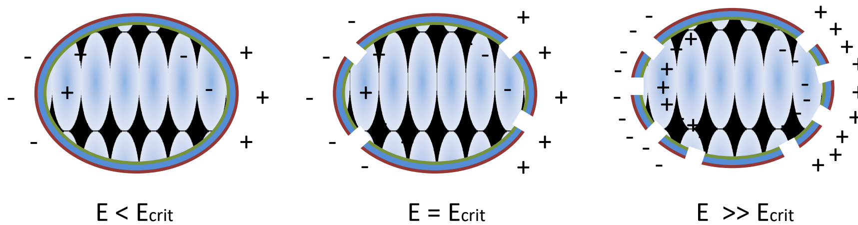

Figure 6. *Mechanism of PEF induced permeabilization.*

Pore formation is a dynamic process that can be either reversible or irreversible depending on the treatment intensity (Zderic et al. 2013). Low treatment intensities induce small pores in comparison to the membrane area, leading to a reversible breakdown. Increasing the intensity of the treatment by increasing the electric field strength and/or treatment time will result in the formation of large pores and reversible permeabilization will turn into irreversible disruption of the cell membrane (Angersbach et al. 2000; Zderic et al. 2013).

Therefore, based on the electrical breakdown of cell walls induced by PEF, different applications of this technology have been developed over the last years.

3.1.2. Food application of PEF treatments

The application of PEF induces structural changes and the rapid breakdown of the cell membrane (Martín-Belloso & Soliva-Fortuny 2011; Toepfl et al. 2005). This process, also called electroporation, has been extensively studied during the last decades (Zimmermann 1986; Vorobiev & Lebovka 2009). The effectiveness of PEF treatments principally depends on the main processing parameters, such as the electric field strength and treatment time, and on the intrinsic factors of the food product, such as the composition and its physical properties (Martín-Belloso & Soliva-Fortuny 2011)

During the last decades, the application of pulsed electric field (PEF) treatments with different intensities has emerged as a promising tool for the food industry. Many studies have been developed with the

objective of inducing microbial inactivation through the application of a high-intensity electric field (Álvarez, Condón, & Raso, 2006; Pagán & Mañas, 2006), as a non-thermal alternative to heat pasteurization. In this regard, extensive research studies have concluded that the application of PEF may assure the inactivation of both pathogenic and spoilage microorganisms, hence maintaining the physicochemical quality of liquid food products without substantially impacting the content and composition of thermolabile compounds (Aguiló-Aguayo et al. 2011; Odriozola-Serrano, Soliva-Fortuny, Hernández-Jover, et al. 2009; Toepfl et al. 2005).

In the last couple of decades, based on the electroporation phenomenon of plant cells, the application of PEF has been proposed as a tool to improve the extraction of intracellular compounds (Vorobiev & Lebovka 2006), to assist drying and freezing processes (Angersbach, Heinz, & Knorr, 1999), to enhance the osmotic dehydration (Ade-Omowaye et al. 2001) as well as to modify enzymatic activity (Martín-Belloso & Elez-Martínez 2005). In addition, the application of low to mild intense PEF treatments has been suggested as a new way to induce stress reactions leading to the biosynthesis of secondary metabolites (Toepfl et al. 2005; Vallverdú-Queralt, Oms-Oliu, et al. 2013; Balasa & Knorr 2011; Vallverdú-Queralt et al. 2012; Balasa 2014; Guderjan et al. 2005). However, PEF-induced responses are not well understood yet due to the complexity of the processes occurring in real food systems.

3.1.3. Plant responses to PEF-induced stress

Some authors have proposed using PEF as an external elicitor for induction of stress reactions in plants. It has been reported that reversible membrane permeabilization induces generation of ROS in plant cells. This fact can lead to the production of secondary metabolites as a plant response under strained conditions (Galindo et al. 2009; Vallverdú-Queralt et al. 2012; Balasa 2014). However, the real mechanisms induced by PEF in the plant cells are still poorly understood.

An hypothetical model describing the generation of ROS in plant cells induced by electropermeabilization has been proposed by Sabri et al. (1996) (Figure 7).

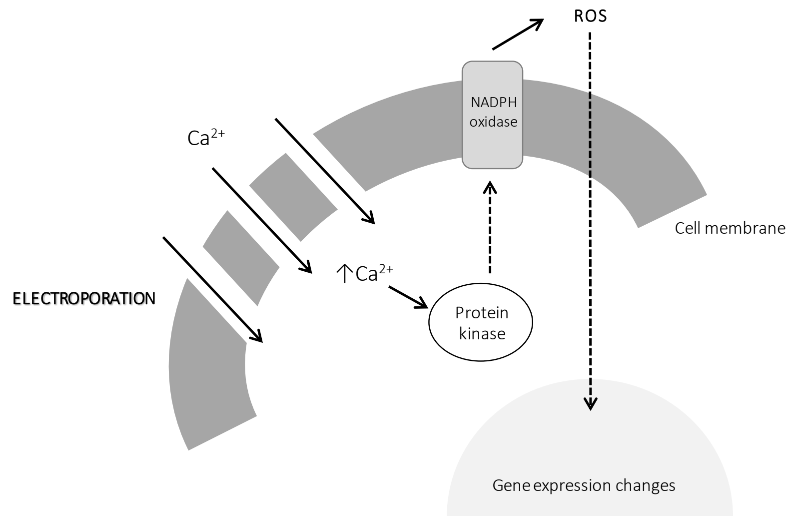

Figure 7. *Model describing the generation of activated oxygen species in plant cells induced by PEF electropermeabilization (adapted from Sabri et al. 1996).*

This model suggests that the cell membrane breakdown could induce a Ca^{2+} influx into cell nucleus. Increased concentration of Ca^{2+} in the intracellular surrounding activates a protein kinase. A membrane bound oxidase (NADPH) is in turn being stimulated by protein kinase, which is responsible for the ROS generation. The excessive production of ROS leads to a progressive oxidative damage by eliciting peroxidation of lipids, oxidation of proteins, damage to nucleic acids, enzyme inhibition, activation of programmed cell death pathway and ultimately cell death (Sharma et al. 2012). In order to overcome these deleterious effects, plants have developed an efficient antioxidative system which allows scavenging or detoxifying the excess of ROS. This system comprises non-enzymatic (ascorbate, glutathione, carotenoids, tocopherols and phenolic compounds) as well as enzymatic antioxidants (superoxide

dismutase, catalase, guaiacol peroxidase, enzymes of ascorbate-glutathione among others) (Hideg et al. 2013).

3.2. Pulsed light treatments

3.2.1. Fundamental aspects of PL processing

Pulse light (PL) is an emerging non-thermal technology which involves the use of intense pulses of short duration and broad spectrum light. It is considered as an alternative to continuous ultraviolet light treatments for solid and liquid foods. The equipment used to deliver PL is composed by one or more inert-gas flash lamps, commonly xenon, a power unit and a high voltage connection (Figure 8).

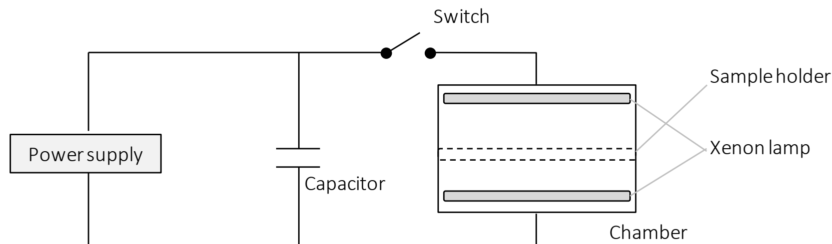

Figure 8. *Schematic circuit of a pulsed light system.*

Pulsed light is produced using technologies that multiply the power manifold (Gómez-López et al. 2007). Electromagnetic energy is accumulated in a capacitor and released in the form of light during a short time, resulting in an amplification of power with a minimum of additional energy consumption (Oms-Oliu, Martín-Belloso, et al. 2010). A typical pulsed light generation system produces a polychromatic radiation within the wavelength range of 180-1100 nm, thus comprising UV-C (200 - 280 nm), UV-B (280-315) nm, UV-A (315 - 400 nm), visible (400 - 700 nm) and infrared (700 - 1100 nm) fractions (Dunn 1996; Demirci & Krishnamurthy 2011). UV radiation is usually the major component of pulsed light. In addition, it is also the most important in terms of energy, compared to visible and infrared regions (Soliva-Fortuny & Martín-Belloso 2016).

3.2.2. Food applications of PL treatments

This technology is commonly used as an emerging non-thermal technology to decontaminate surfaces by microbial inactivation (Gómez-López et al. 2007; Oms-Oliu, Martín-Belloso, et al. 2010; Dunn 1996). PL processing was adopted by the food industry since 1996, when the Food and Drug Administration (FDA) approved its use for production, processing and handling of foods (FDA 2016).

PL has been shown to be effective in inactivating a wide broad of microorganisms involved in food spoilage and foodborne pathogens. The mechanisms of microbial inactivation proposed to explain the lethal effect are related to the UV part of the spectrum and have been reported to have a photochemical and a photothermal nature (Oms-Oliu, Martín-Belloso, et al. 2010). High content in UV wavelengths produces DNA damage (e.g. formation of pyrimidine dimers and other photoproducts), thus leading to antimicrobial inactivation. In this regard, extensive research studies have proposed the exposure to UV treatment as a feasible technology to disinfect water, air and food contact surfaces (Luksiene et al. 2012; Gómez-López et al. 2007; Oms-Oliu, Martín-Belloso, et al. 2010).

In the last years the application of PL treatments is arousing increasing interest in food processing for its ability to improve the beneficial properties of fruits and vegetables for human health by increasing the amount of some phytochemicals (Bravo et al. 2012; Charles et al. 2013; Lopes et al. 2016). In this regard, Bravo et al. (2012) noticed that plant hormesis induced by UV-C postharvest treatments significantly enhanced the content of bioactive compounds (lycopene and total phenolic compounds) and the antioxidant activity of tomato fruits. In addition, Liu et al. (2011) found that UV-B irradiation promoted the accumulation of total flavonoids and total phenolic compounds in tomato fruits over storage. Furthermore, Pataro et al. (2015) concluded that pulsed light treatments are effective in activating the biosynthetic pathways of both carotenoids and phenolic compounds in tomato fruits.

3.2.3. Plant responses to PL-induced stress

Carotenoid metabolism in tomato fruits may be affected by environmental factors, such as radiation intensity and temperature, among others (Liu et al. 2015). After harvest, the effect of the exposure of tomatoes to high light intensity appears to be positive for some antioxidant compounds (Poiroux-Gonord et al. 2010). Gautier et al. (2008) reported that tomato irradiance enhanced the first step of carotenogenesis by modulating the phytoene synthase activity, which is an important control step of carotene biosynthesis. In addition, it has been described that UV-radiation could induce specific changes in gene expression, alter phytochemical contents and increase the accumulation of UV-screening pigments (Hideg et al. 2013). These changes are related with the increased generation of ROS, which exhibit several deleterious effects in plants (Lopes et al. 2016; Sharma et al. 2012). However, the activation of defense system of plants could be activated for scavenging the generated ROS. The defense system is composed of nonenzymatic and enzymatic antioxidants. Nonenzymatic antioxidants such as ascorbate, carotenoids, tocopherols and phenolic compounds, act as redox buffers and reduce the harmful effect of ROS in plant cells (Sharma et al. 2012; Hideg et al. 2013). Some studies have reported a significant enhancement in these bioactive compounds after different light treatments which was related to a protective response of plants against an abiotic stress (Lopes et al. 2016; Aguiló-Aguayo et al. 2013; Liu et al. 2009; Pataro et al. 2015; Charles et al. 2013).

4. Final remarks

Consumers have switched from an emphasis on satisfying hunger to an emphasis on the promising use of foods to promote well-being and to help reducing the risk of diseases. To this purpose, there is a strong research activity in investigating new strategies to provide food products with high health-related properties. Therefore, the development of new processing technologies that can result in maximum overall concentration and bioaccessible fraction of

carotenoids in tomato and tomato-based products has become a thrilling challenge for food scientists and technologists.

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OBJECTIVES

The main objective of this Doctoral Thesis was to enhance the concentration and bioaccessibility of carotenoids in tomato and tomato-based products through the application of non-thermal technologies of food processing. To achieve this main goal, the following specific objectives were proposed:

- ✓ To evaluate the feasibility of using PEF treatments for enhancing the carotenoids concentration in tomato fruits; firstly by investigating the effect of PEF processing conditions on the carotenoids content as well as on the respiratory activity and the main physicochemical properties of the fruits; and eventually, by studying the effect of post-treatment storage conditions on the carotenoid profile and quality attributes of tomato.

- ✓ To study the effect of postharvest PL treatments of different spectral range on the carotenoids concentration as well as on the main physicochemical properties of tomato fruits during storage.

- ✓ To assess the effect of the ripeness stage as well as the addition of different types of oil on the concentration and bioaccessibility of carotenoids of different tomato-based products.

- ✓ To evaluate the application of PEF to tomato fruits in order to enhance the concentration and the bioaccessible fraction of carotenoids in a subsequently obtained derived product.

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1. Experimental design

This section details the experimental setup followed to achieve the main research objectives of the current Doctoral Thesis.

EXPERIMENT 1. Enhancing the carotenoid content of tomato fruit with pulsed electric field treatments: effects on respiratory activity and quality attributes.

In this study, the application of pulsed electric field (PEF) treatments as an abiotic elicitor was assessed in order to enhance the carotenoids content of tomato fruits as a response of fruits to stress. This experiment was aimed at evaluating the changes in the quality attributes and respiratory activity in tomato fruits associated to the enhancement of carotenoids concentration when different PEF treatments were applied.

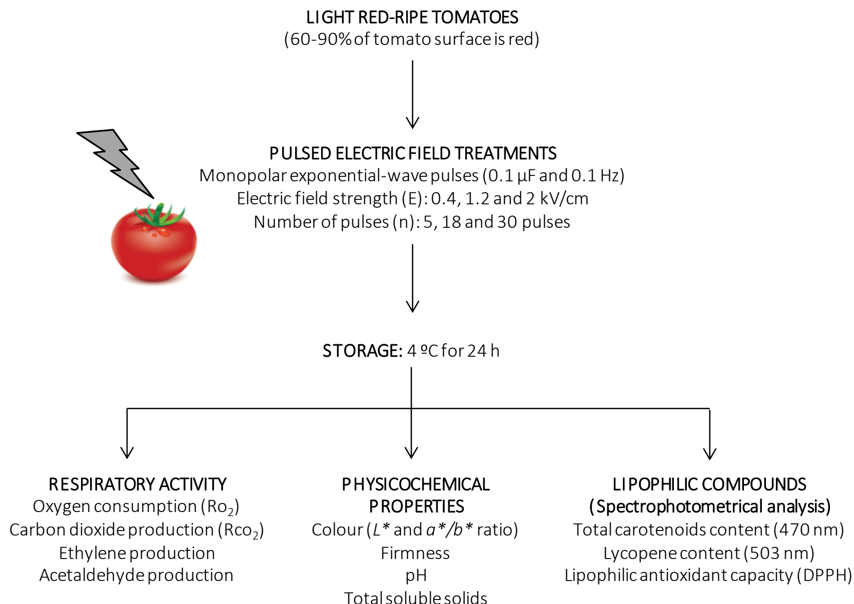


Figure 1. *Work plan to assess the effect of stress-inducing pulsed electric fields treatments on the carotenoids content, respiratory activity and quality attributes of tomato fruits.*

EXPERIMENT 2. Evaluation of physicochemical properties and carotenoid profile of tomato fruits during storage at different temperatures after pulsed electric fields processing.

Based on the results obtained in experiment 1, tomatoes were subjected to different PEF treatments in order to obtain tomato fruits with higher amount of carotenoids. The influence of the post-treatment storage conditions (time and temperature) on the carotenoid profile as well as on the main physicochemical properties of tomato was assessed.

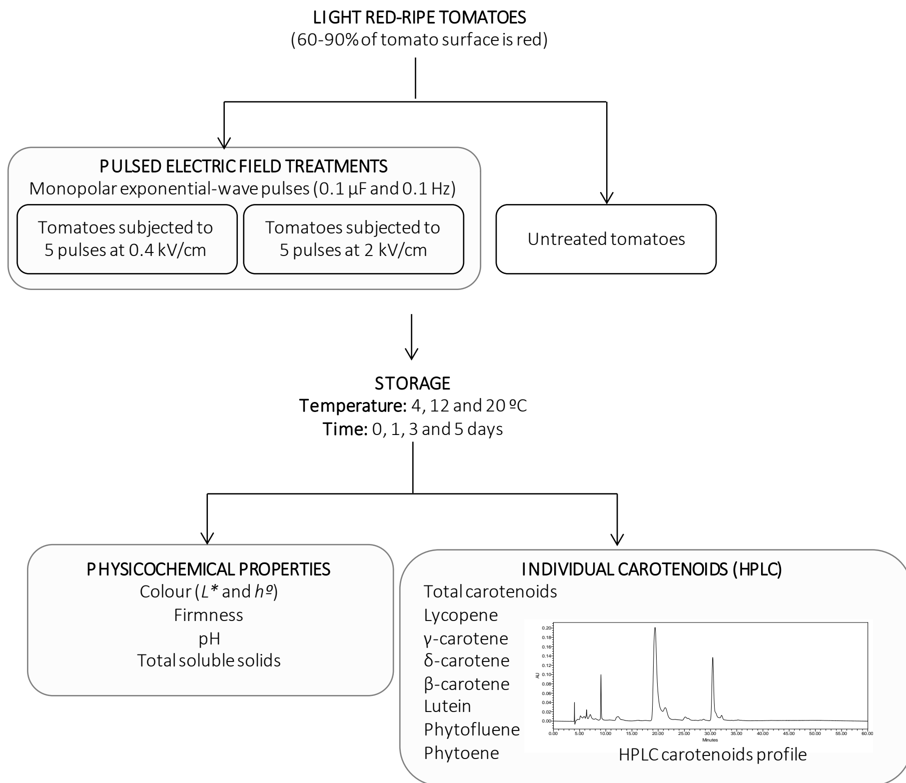


Figure 2. *Work plan to evaluate the physicochemical properties and carotenoid profile of tomato fruits during storage at different temperatures after pulsed electric fields processing.*

EXPERIMENT 3. Influence of pulsed light dose spectral range on the accumulation of carotenoids and quality attributes of tomato fruits during post-treatment storage.

The application of pulsed light treatments is also proposed as an innovative strategy to obtain tomatoes with an enhanced amount of carotenoids, especially lycopene. The effect of the spectral distribution (full spectrum light, full spectrum without UV-C light and VIS-NIR light) on the accumulation of carotenoids and on the main quality characteristics of tomato fruits was assessed during post-treatment storage.

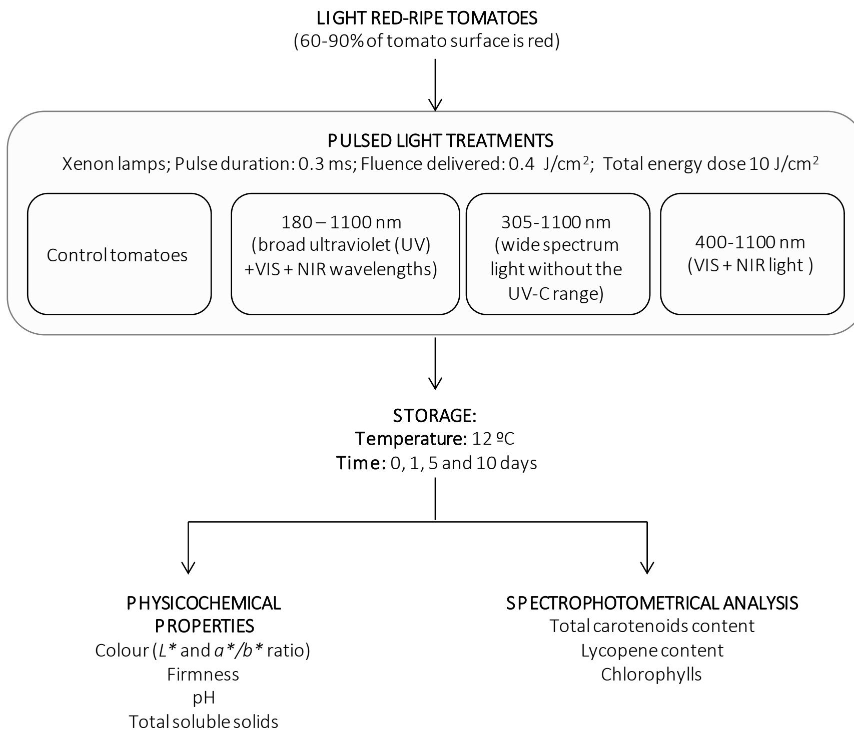
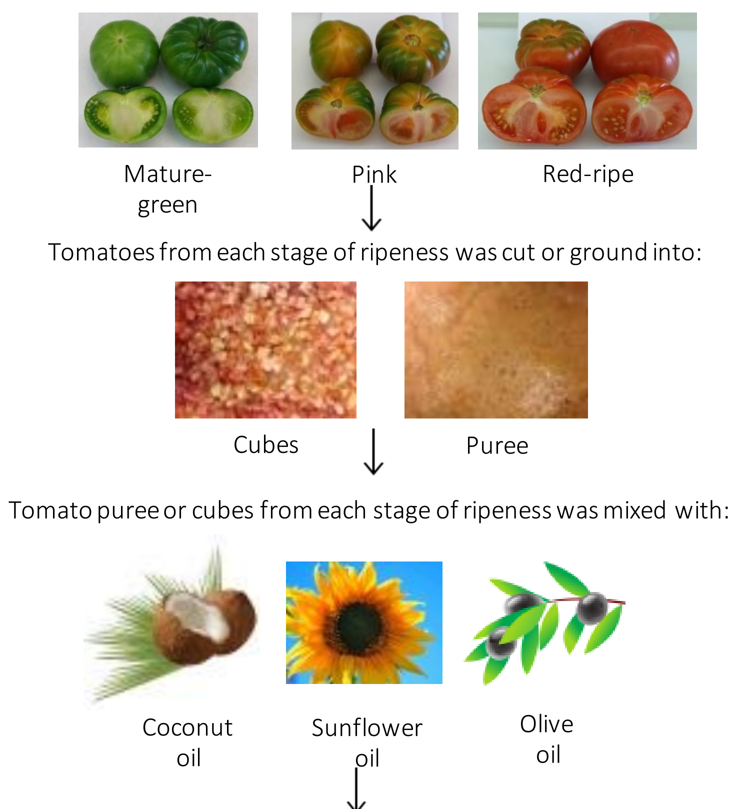


Figure 3. *Work plan to study the influence of pulsed light dose spectral range on the accumulation of carotenoids and quality attributes of tomato fruits.*

EXPERIMENT 4. In vitro bioaccessibility of carotenoids in tomato derivatives as affected by ripeness stage and the addition of different types of oil.

This work was carried out in order to assess the simultaneous effect of tomato ripeness stage (mature green, pink and red-ripe), mechanical processing (cutting and grinding) and oil addition (coconut, sunflower, and olive oils) on the amount and bioaccessible fraction of carotenoids. A static *in vitro* gastrointestinal digestion consisting of oral, gastric and small intestinal phases was simulated. Total carotenoids and lycopene concentrations were determined spectrophotometrically before and after the *in vitro* digestion. Bioaccessibility values of total carotenoids and lycopene were evaluated.



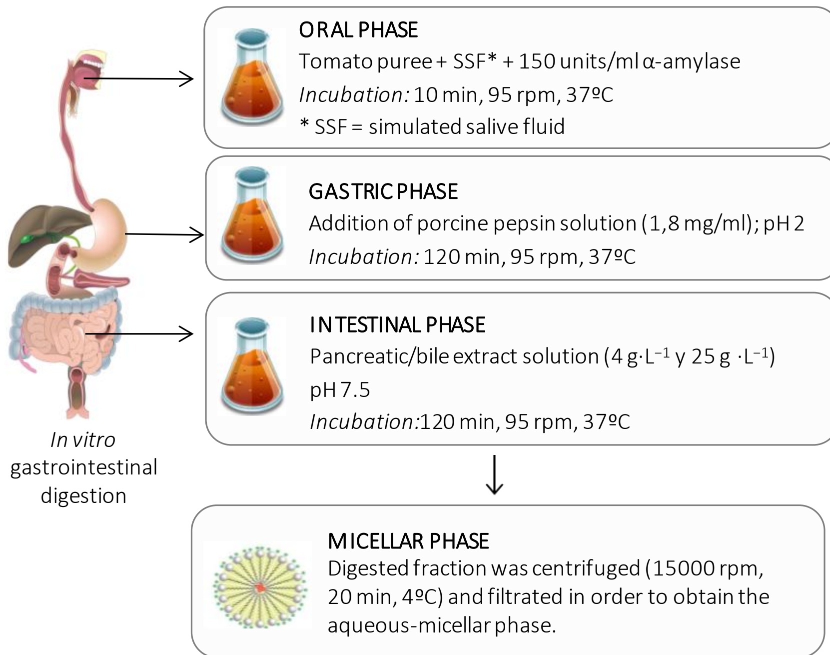


Figure 4. *Work plan to determine the in vitro bioaccessibility of carotenoids in tomato derivatives as affected by ripeness stage and the addition of different types of oil.*

EXPERIMENT 5. Application of pulsed electric fields to tomato fruits for enhancing the concentration and bioaccessible fraction of carotenoids in a derived product.

The application of moderate intensity pulsed electric fields to tomato fruits was proposed as a pre-processing treatment in order to obtain tomato purees with increased amount of carotenoids. The effect of the PEF processing conditions (electric field strength and number of pulses) on the concentration as well as on the bioaccessible fraction of individual carotenoids of tomato puree with 5% of added olive oil was assessed.

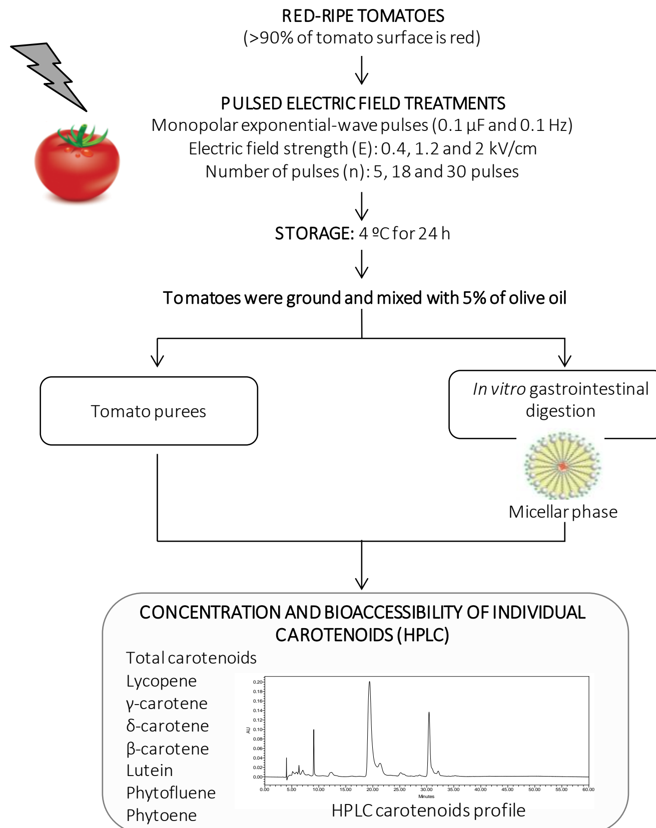


Figure 5. *Work plan to evaluate the effect of applying pulsed electric fields to tomato fruits on the concentration and bioaccessible fraction of carotenoids in a derived product.*

2. Detailed methodologies

2.1. Materials

2.1.1. Raw tomatoes

Raw tomatoes (*Lycopersicum esculentum* cv. Raf) were purchased in a local market (Lleida, Spain). According to USDA (1991), the following terms describe the stage of ripeness used in this Doctoral Thesis depending on the experiment carried out:

- Mature-green stage: the fruit cuticle is completely green in color, varying from light to dark green.
- Partially ripe: more than 30% but not more than 60% of the surface shows red color.
- Light-red stage: more than 60% but not more than 90% of the surface shows red color.
- Fully ripe: more than 90% of the surface exhibits red color.

Once in the laboratory, tomatoes were placed in a cool chamber at 12 ºC and stored until processing.

Prior to processing, tomatoes with uniform shape and size, free from fungal infection, were selected. Fruits were rinsed with tap water and carefully dried with paper cloth to remove adhering dirt on their surface.

2.1.2. Tomato derivatives

Two different tomato-based products were studied in this thesis. On the one hand, tomato cubes were obtained by cutting the fruits approximately into 1-cm^3 pieces. Afterwards, they were mixed with 5% of different types of oil (coconut, olive and sunflower oil). On the other hand, a 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. Tomato derivatives without oil were also prepared as control.

2.1.3. Reagents

α-amylase, pepsin from hog stomach, pancreatin from porcine pancreas, bile extract porcine, DPPH (2,2-diphenyl-1-picrylhydrazyl), Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), magnesium hydroxide carbonate, calcium chloride dihydrate, magnesium chloride hexahydrate (99%), magnesium sulfate 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). Lycopene, γ-carotene, δ-carotene, β-carotene, lutein, phytofluene and phytoene were obtained from Carote-Nature (Ostermundigen, Switzerland).

2.2. Non-thermal technologies

2.2.1. Pulsed electric fields (PEF) treatments

2.2.1.1. PEF pilot plant equipment

During the course of this thesis, PEF treatments were conducted in a batch lab scale system manufactured by Physics International (San Leandro, CA, USA). The apparatus delivers monopolar exponential-wave pulses from a capacitor of 0.1 μF with a frequency of 0.1 Hz. The treatment chamber consists of a rectangular methacrylate container with two parallel stainless steel electrodes separated by a gap of 10 cm. A batch of tomato fruits was placed into the treatment chamber and filled with tap water. Each treatment was applied to two fruits at a time. Different electric field strengths and number of pulses were applied to whole tomato fruits.

The specific energy input corresponding to each treatment was calculated following equation 1, according to Luengo, Condón-Abanto, Álvarez, & Raso (2014):

$$Q\left(\frac{J}{g}\right) = \frac{V^2 * Co * n}{2\ m} \quad (1)$$

Where, V is the charging voltage (V), C_o is the capacitance (F), n is the number of pulses and m is the mass of product (g).

The equivalences are displayed in Table 1.

Table 1. *PEF treatment conditions and the calculated specific energy input.*

Electric field strength (kV·cm^{-1})	Number of pulses	Specific energy input (kJ·kg^{-1})
0	0	Untreated
0.4	5	0.02
0.4	18	0.06
0.4	30	0.09
1.2	5	0.14
1.2	18	0.50
1.2	30	0.83
2	5	0.38
2	18	1.38
2	30	2.31

2.2.2. Pulsed light (PL) treatments

2.2.2.1. PL plant equipment

Pulsed light (PL) treatments were carried out using a XeMaticA-2L system (SteriBeam Systems GmbH, SteriBeam, Kehl, Germany) equipped with two Xenon lamps located above and below the sample holder, separated by a gap of 17 cm. The emitted spectrum wavelengths ranged from 180 to 1100 nm, with 15-20 % of the light in

the UV region. The pulse duration was 0.3 ms. The fluence delivered by each lamp was 0.4 $J\cdot cm^{-2}$ per pulse. Different PL doses (2, 10 and 25 $J\cdot cm^{-2}$) were applied to whole tomatoes. These PL doses were attained by the application of different number of pulses (5, 25 and 63 pulses, respectively). To evaluate the effect of the application of light pulses with different spectrum composition, two types of filters were used: a Makrolon® polycarbonate filter (MF) which cuts off all light below 400 nm, thus allowing only the VIS and NIR to pass through, and a 2-mm thick Pyrex® glass filter (PF) that cuts all light below 305 nm allowing to pass UV-B (280-320nm), UV-A (320-400 nm), VIS and NIR wavelengths. Additionally, treatments with no filter (FS) were carried out to assess the effect of full emitted spectrum (180-1100 nm).

2.3. *In vitro* gastrointestinal digestion

A static *in vitro* gastrointestinal digestion was carried out in order to determine the bioaccessibility of carotenoids of tomato-based products. The *in vitro* digestion was carried out following the methodology proposed by Rodríguez-Roque et al. (2013) with slight modifications, which consists of an oral, a gastric and a small intestinal phase.

Oral phase: a portion of 75 grams of tomato product (cubes or puree) was weighed and mixed with 75 mL of simulated salivary fluid (SSF), which contained 150-200 $U\cdot mL^{-1}$ of α-amylase (Tagliazucchi et al. 2012). The composition of SSF was 0.1854 $g\cdot L^{-1}$ of $CaCl_2\cdot 2H_2O$, 0.4 $g\cdot L^{-1}$ of KCl, 0.06 $g\cdot L^{-1}$ of KH_2PO_4, 0.1 $g\cdot L^{-1}$ of $MgCl_2\cdot 6H_2O$, 0.049 $g\cdot L^{-1}$ of $MgSO_4\cdot 7H_2O$, 8 $g\cdot L^{-1}$ of NaCl, 0.35 $g\cdot L^{-1}$ of $NaHCO_3$ and 0.048 $g\cdot L^{-1}$ of Na_2HPO_4 (pH = 6.8). The mixture was homogenized in a stomacher laboratory blender (IUL Instruments, Barcelona, Spain) for 1 min in order to simulate mastication. Subsequently, it was incubated in an orbital shaker (Ovan, Badalona, Spain) at 37 ºC for 10 min and 95 rpm (Tagliazucchi et al. 2012).

Gastric phase: pH of the digesta was adjusted to 4 with 1 M HCl. Then, a porcine pepsin solution from hog stomach (40 $g\cdot L^{-1}$ in 0.1 M HCl) was

added to assure a final concentration of 1.8 g·L^{-1} in the gastric digesta. The pH was immediately adjusted to 2 by adding 5 M HCl. The mixture was incubated at 37 °C and 95 rpm for 120 h in an orbital shaker.

Small intestinal phase: to simulate duodenal conditions, pH of the digesta was set to 5.3 with 2 M NaOH. Then, 15 mL of pancreatin/bile solution (4 g·L^{-1} and 25 g·L^{-1} in 0.1 M $NaHCO_3$ (w/v), respectively) were added into the small intestinal digesta. The pH was then immediately adjusted to 7.5 with 2 M NaOH. The mixture was incubated at 37 ºC and 95 rpm for 120 min in an orbital shaker.

To quantify the amount of carotenoids released from the tomato matrix and incorporated into the micellar fraction, the small intestinal digest was centrifuged at 33.768 g during 20 min at 4°C (Beckman Coulter, Avanti J-26 XP, California, United States). The aqueous-micellar phase was collected and filtered through no.1 Whatman filter paper and then, across a cellulose filter (1-3 µm pore size, 70 mm diameter, Filtros Anoia S.A., Barcelona, Spain) in order to eliminate any crystalline carotenoid or undigested lipid. Finally, the micellar phase was freeze-dried and stored at -40 ºC until carotenoids extraction.

2.3.1. Bioaccessibility calculations

Bioaccessibility was determined through equation 2. Results were expressed as the percentage of carotenoids transferred from tomato matrix to the micellar fraction after the *in vitro* digestion.

$$Bioaccessibility\ (\%) = \frac{CC_{digested}}{CC_{undigested}} x100 \qquad (2)$$

where $CC_{digested}$ corresponded to the overall concentration of carotenoids in the micellar fraction and $CC_{undigested}$ was the concentration in the non-digested samples.

2.4. Physicochemical characterization of tomato fruits

2.4.1. Colour

The external colour of tomato fruits was characterized using a Minolta colorimeter (Minolta CR-400, Konica Minolta Sensing, Inc., Osaka, Japan). The equipment was set up for a D65 illuminant and 10º observer angle. A white standard plate (Y = 94.00, x = 0.3158, y = 0.3322) was used for calibration. The CIELab colour space coordinates (lightness, L*; green-red chromaticity, a*; and blue-yellow chromaticity, b*) were measured. The variation of tomato colour was assessed by determining the lightness (L*), the a*/b* ratio and the hue angle (hº), which is calculated following equation 3.

$$ºh = tan^{-1} \frac{b^*}{a^*} \quad (3)$$

2.4.2. Firmness

Firmness was determined with a TA-XT2 texture analyzer (Stable Micro Systems Ltd., Surrey, England) by measuring the maximum force required to penetrate tomato tissue to a depth of 10 mm using a 4-mm-diameter steel probe at a shearing speed of 5 $mm \cdot s^{-1}$. Results were expressed in Newtons (N).

2.4.3. pH

Previous to pH measurements, tomatoes were ground in a blender in order to obtain a homogeneous sample, which was used for determining the pH values. The pH was measured using a Crison 2001 pH-meter (Crison Instruments S.A., Alella, Barcelona, Spain) at 25ºC.

2.4.4. Total soluble solids content (TSS)

The same homogeneous sample obtained to measure the pH of tomatoes was also used to determine the total soluble solids content. TSS was determined by measuring the refraction index with an Atago

RX-1000 refractometer (Atago Company Ltd., Tokyo, Japan) at 25 ºC. The results were expressed as ºBrix.

2.4.5. Titratable acidity

Titratable acidity was estimated after titration at pH 8.1 with 0.1 N NaOH. Results were expressed as grams of citric acid·kg^{-1}.

2.5. Respiratory activity

The respiratory activity of tomatoes was determined using a static system. Tomato fruits (ca. 130 g) were individually placed in hermetic containers (0.5 L of capacity). Changes in the composition of the headspace were measured using a gas analyzer (Varian 490 Micro-GC, Middelburg, The Netherlands). A 1.7 mL sample was withdrawn from the headspace atmosphere through an adhesive rubber septum with a syringe. Portions of 0.25 and 0.33 mL were injected for O_2 and CO_2 determination, respectively. The O_2 content was analysed with a CP-Molsieve 5Å column (10 m x 0.32 mm, df = 30 µm) at 60 ºC and 100 kPa. For quantification of CO_2, ethylene (C_2H_4) and acetaldehyde (C_2H_4O), a Pora-PLOT Q column (10 m x 0.32 mm, df = 10 µm) at 70 ºC and 200 kPa, was used. Both columns were equipped with a thermal conductivity detector. The oxygen consumption (Ro_2) and carbon dioxide production (Rco_2) were determined according to Fonseca, Oliveira, & Brecht (2002) (eq. 4 and 5, respectively):

$$Ro_2 = \frac{\left(o_2^i - o_2^f\right) x\, V}{m\, x\, (t_f - t_i)} \qquad (4)$$

where O_2^i is the initial oxygen concentration; O_2^f is the final oxygen concentration; m is the tomato weight (g) and $(t_f - t_i)$ is the time of storage.

$$Rco_2 = \frac{\left(co_2^f - co_2^i\right) x\, V}{m\, x\, (t_f - t_i)} \quad (5)$$

where CO_2^f is the final carbon dioxide concentration; CO_2^i is the initial carbon dioxide concentration; m is the tomato weight (g) and $(t_f - t_i)$ is the time of storage. Results were expressed as mg·h^{-1}·kg^{-1} (fw).

In addition, ethylene and acetaldehyde production were analyzed and expressed as ng·h^{-1}·kg^{-1} (fw).

2.6. Analytical methods

2.6.1. Total carotenoids and lycopene analysis: spectrophotometrical analysis

Total carotenoids (TCC) and lycopene (LC) concentration was quantified spectrophotometrically.

2.6.1.1. Extraction

Lipophilic extracts were obtained following the methodology reported by Odriozola-Serrano, Aguiló-Aguayo, Soliva-Fortuny, Gimeno-Añó, & Martín-Belloso (2007) with slight modifications. Freeze-dried tomato samples (0.2 g) were weighed and mixed with 20 mL of 1% (w/v) of butylated hydroxytoluene (BHT) in ethanol:hexane (4:3 v/v). The mixture was homogenized at 200 rpm, for 15 min and 4 ºC in a Beckman Coulter centrifuge (Avanti J-26 XP, California, United States). Then, 3 mL of distilled water were added and the mixture was shaken and kept at room temperature for 5 minutes to allow phase separation. The lipophilic extracts were used to measure the total carotenoids and lycopene concentration, as well as the lipophilic antioxidant activity. All procedures were performed in dim lighting in order to prevent carotenoids photodegradation.

2.6.1.2. Total carotenoids determination

Total carotenoids content (TCC) was determined spectrophotometrically following the methodology proposed by Talcott & Howard (1999). The absorbance of the organic phase was measured at 470 nm versus a blank of hexane. TCC was calculated using the following equation (6):

$$\textit{Total carotenoids content } (mg \cdot kg^{-1}) = \frac{A_{470}\ x\ V\ x\ 10^4}{A_{1cm}^{1\%}\ x\ G} \quad (6)$$

where A_{470} is the absorbance at 470 nm, V is the total volume of extract (mL), $A_{1cm}^{1\%}$ is the extinction coefficient of a mixture of carotenoids established in 2500 by Gross (1991) and G is the sample weight (g). Total carotenoids were expressed as $mg \cdot kg^{-1}$ of fresh weight (fw).

2.6.1.3. Lycopene quantification

The absorbance of the lipophilic extract was measured at 503 nm using hexane as a blank. Lycopene concentration was calculated following the methodology proposed by Fish, Perkins-Veazie, & Collins (2002) (equation 7).

$$\textit{Lycopene concentration } (mg \cdot kg^{-1}) = \frac{A_{503}\ x\ MW\ x\ DF\ x\ 10^6}{\varepsilon\ x\ L} \quad (7)$$

where A_{503} is the absorbance at 503 nm, MW is the molecular weight of lycopene (536.9 $g \cdot mol^{-1}$), DF is the dilution factor, ε is the molar extinction coefficient for lycopene (17.2 x 10^4 $L \cdot mol^{-1} \cdot cm^{-1}$) and L is the pathlength. Lycopene concentration was expressed as $mg \cdot kg^{-1}$ (fw).

2.6.1.4. Lipophilic antioxidant capacity

Lipophilic extracts prepared to determine total carotenoids and lycopene contents were also used to analyze the lipophilic antioxidant capacity (LAC). LAC was evaluated using the colorimetric method reported by Vallverdú-Queralt et al. (2012) which is based on the free

radical scavenging effect of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical. Ten microliters of tomato extract were mixed with 90 µL of distilled water and 3.9 mL of $DPPH^{\cdot}$ solution. The mixture was shaken vigorously in a vortex and kept in the dark for 30 min. The absorbance was measured at 515 nm. Results were compared with a standard curve prepared with Trolox and expressed as µmol Trolox equivalents $(TE)\cdot kg^{-1}$ (fw).

2.6.1.5. Chlorophylls analysis

The extraction of chlorophylls was carried out following the methodology proposed by Costache, Campeanu, & Neata (2012), with slight modifications. Freeze-dried tomato samples (0.2 g) were mixed and homogenized with 20 mL of 100% acetone in an Ultraturrax (T-25 Basic, IKA®-Werke GmbH & Co., Staufen, Germany) for 2 minutes in an ice-bath. Homogenized mixture is separated by centrifugation (Beckman Coulter, Avanti J-26 XP, California, United States) at 3000 g for 10 minutes at 4 ºC. The extract was then filtered a Whatman no. 1 paper. The extract was transferred to a 25 mL flask and the volume was adjusted with acetone. The analytical determination was performed spectrophotometrically (CECIL CE 2021; Cecil Instruments Ltd., Cambridge, UK) at the following wavelengths: 662 nm, 645 nm and 470 nm for chlorophyll *a,* chlorophyll *b* and carotenoids, respectively. The total carotenoids concentration was calculated following Eq. 8, Eq. 9 and Eq. 10. Total chlorophylls content was calculated as the sum of chlorophyll *a* and chlorophyll *b.* Results were expressed as $mg\cdot kg^{-1}$(fw).

$$C_a = 11.75\ A_{662} - 2.35\ A_{645} \quad (8)$$

$$C_b = 18.61\ A_{645} - 3.96\ A_{662} \quad (9)$$

$$C_c = (1000\ A_{470} - 2.27\ C_a - 81.4\ C_b)/227 \quad (10)$$

where C_a is the content of chlorophyll *a*, C_b the content of chlorophyll *b*, and C_c the total carotenoids concentration.

2.6.2. Individual carotenoids

2.6.2.1. Extraction

Carotenoid extraction was carried out following the methodology proposed by Rodríguez-Roque, et al. (2013) with slight modifications. Freeze-dried tomato sample was mixed with 0.1% of magnesium hydroxide carbonate (w/w) and 10 mL of 0.05% (w/v) BHT in ethanol:hexane (4:3 v/v). The mixture was homogenized using an Ultraturrax (T-25 Basic, IKA®-Werke GmbH & Co., Staufen, Germany) for 2 min in an ice-bath. It was then filtered under vacuum through no. 1 Whatman paper. The residue was re-extracted once with 10 mL of ethanol:hexane (4:3 v/v) for 2 min with the Ultraturrax. Then, the mixture was again filtered and the residue was washed twice with 5 mL of ethanol and once with 5 mL of hexane. All the filtrates were combined in an amber round-bottom flask and rotoevaporated (rotovapor R-3000, BUCH, Switzerland) at 45 ºC for 15 min to dryness. The residue was then saponified under a N_2 atmosphere by adding 10 mL of methanolic KOH 0.5 M + 0.1% BHT (v/w) and 10 mL of diethyl ether for 30 min with continuous agitation. Afterwards, the extract was placed in an amber decanting funnel and washed twice with 25 mL of 10% NaCl solution and thrice with 25 mL of distilled water. The aqueous phase was discarded each time. The organic phase was collected and rotoevaporated at 45 °C for 20 min to dryness. The residue was dissolved with 4 mL of diethyl ether and placed in an amber glass vial. Finally, the solvent was evaporated under a N_2 flow and stored at −40°C until analysis. Before injection into the HPLC system, the carotenoid extract was reconstituted with 1 mL of methylene chloride and filtered through a 0.45 µm filter.

2.6.2.2. Quantification

Carotenoids were quantified by high-performance liquid chromatography (HPLC) following the methodology reported by Odriozola-Serrano et al. (2009). The HPLC system was equipped with a 600 controller and a diode array detector 2996 (Waters Corp.) which was set to scan from 240 to 550 nm. Separations were performed on a

reverse-phase C18 Spherisorb® ODS2 (5 µm) stainless steel column (4.6 mm x 250 mm) at room temperature with a flow rate of 0.7 mL·min^{-1}. An isocratic elution of acetonitrile (85%), methanol (10%), methylene chloride (3%) and hexane (2%) was maintained from 0 to 10 min, followed by a linear gradient to acetonitrile (45%), methanol (10%), methylene chloride (23%) and hexane (22%) from 10 to 40 min. Finally, the initial conditions were re-established for 20 min. Individual carotenoids were identified by their retention time, absorption and spectra, according to Khachik et al. (1992) (Table 2).

Table 2. *HPLC peak identification of the carotenoids in tomato (Adapted from Khachik et al., 1992).*

Carotenoid	Wavelength (nm)	Retention time (min)
Lutein	446	6-7
Lycopene	472	20-21
γ-carotene	461	27-28
δ-carotene	402	31-33
β-carotene	454	30
Phytofluene	350	32-33
Phytoene	286	36-37

Carotenoids quantification was carried out by comparison with external standards of lycopene, γ-carotene, δ-carotene, β-carotene, lutein, phytofluene and phytoene. The content for each carotenoid compound was expressed as µg·kg^{-1} (fw). Total carotenoid concentration was calculated as the sum of individual compounds and also expressed as µg·kg^{-1} (fw).

2.7. Statistical analysis

Statistical analyses were carried out using the JMP Pro v.12.0.1 software (SAS Institute, Cary, NC, USA). Results were reported as the mean ± standard deviation. Results were subjected to a factorial analysis of variance (ANOVA) followed by Tukey–Kramer post hoc test in order to establish statistical differences among mean values. The

relationship between variables was determined using the Pearson correlation coefficient. The statistical significance level was set up at $p < 0.05$.

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RESULTS

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CHAPTER 1

Enhancing the carotenoid content of tomato fruit with pulsed electric field treatments: effects on respiratory activity and quality attributes

ABSTRACT

Pulsed electric field (PEF) may be used to elicit the accumulation of carotenoids in plant tissues. The stress-adaptive response to PEF is dependent on the treatments conditions and could lead to undesirable effects on the final quality of tomato fruit. This study was aimed at assessing the changes in the respiratory activity and the main quality attributes of tomato fruit when PEF treatments were used to elicit an increased concentration in their carotenoids content. Whole tomatoes (cv. Raf) were subjected to different electric field strengths (0.4, 1.2 and 2 kV·cm^{-1}) and number of pulses (5, 18 and 30 pulses). After being treated, the fruit were immediately stored at 4 ºC for 24 h. Total carotenoids and lycopene concentrations were enhanced by 50 % and 53 %, respectively, after applying 30 pulses at 2 kV·cm^{-1}. Concurrently, a significant improvement in lipophilic antioxidant capacity was observed. At such treatment conditions, a deceleration in the R_{O_2} and R_{CO_2}, a drop in the ethylene production and the induction of acetaldehyde synthesis were observed as an evidence of the stress injury caused to tomato tissues. In addition, several quality attributes of tomato were significantly affected. Tomatoes subjected to 2 kV·cm^{-1} exhibited the greatest values of total soluble solids and pH, as well as a marked reddening and softening of the fruit. Results suggest that selected PEF conditions could be proposed as a pre-processing treatment to produce tomato-based products with enhanced carotenoid contents.

1. Introduction

Tomato is one of the most important worldwide agricultural crops (Ilahy et al. 2011). Epidemiological studies have shown that the increased consumption of tomato and tomato-based products may reduce the risk of cardiovascular diseases, certain types of cancer and atherosclerosis (Hedges & Lister 2005). These chronic diseases are associated with an excess of reactive oxygen species (ROS), which are capable of inducing cellular damage (Choudhury et al. 2013). Tomato and tomato products are rich in some phytochemical compounds with high free-radical scavenging and quenching capacities (Odriozola-Serrano et al. 2008). These beneficial properties have been attributed in part to carotenoids, especially to lycopene, which is the most abundant carotenoid in red-ripe tomatoes (Dannehl et al. 2010). The accumulation of carotenoids in tomato normally occurs during ripening. However, carotenoid production has been recently reported to be promoted by enzymatically-mediated softening phenomena triggered by ROS generated upon exposure to oxidative stress (Fanciullino et al. 2014).

Consumers are increasingly demanding high quality and healthy products. Therefore, it is important to develop new technologies that allow obtaining products with high antioxidant properties. During the last decades, it has been demonstrated that the application of PEF with different intensities could offer useful applications for the food industry, for instance the inactivation of microorganisms (Álvarez et al. 2006) and quality-related enzymes (Martín-Belloso & Elez-Martínez 2005), the improvement of the extraction of intracellular metabolites (Luengo, Álvarez, et al. 2014), the enhancement of osmotic dehydration processes as well as the assistance of freezing and drying processes (Toepfl et al. 2005; Soliva-Fortuny et al. 2009; Barba et al. 2015). In addition, several research works have reported the feasibility of PEF treatments to stimulate the biosynthesis of defensive secondary metabolites in fruit, such as polyphenols and carotenoids (Balasa & Knorr 2011; Vallverdú-Queralt, Oms-Oliu, et al. 2013; Soliva-Fortuny et al. 2017). It has been suggested that PEF may trigger an oxidative burst

with the consequent production of ROS (Galindo et al. 2009). The bioproduction of secondary metabolites has been related to the ability of plants to overcome unfavourable conditions (Sharma et al. 2012). In this regard, Vallverdú-Queralt et al. (2013a, 2013b) reported a significant improvement in carotenoids and phenolic compounds in whole tomatoes after the application of PEF treatments which was attributed to the activation of some metabolic pathways and to the permeabilization of cellular membranes. Besides producing several changes in metabolism of metabolically-active plants, PEF treatments could induce the modification of respiration rate in plants. Some authors have reported that the respiratory activity of plants was increased by the application of abiotic stress, such as wounding, water deficiency and salinity (Łukaszuk, E. & Ciereszko 2012; Fraire-Velazquez & Emmanuel 2013; Jacobo-Velázquez et al. 2011; Galindo et al. 2007). However, literature data concerning the PEF-induced changes in respiration rate in whole fruit and vegetables are not available.

In concomitance with the acceleration of tomato metabolism after the application of PEF, several changes in quality attributes may be affected. It is known that PEF can strongly affect the tissue firmness of fruit and vegetables, such as carrots, potatoes and apples, because of its action at the cell membrane level (Lebovka et al. 2004; Shayanfar et al. 2013). This fact could lead to undesirable effects on the final quality of tomato. Moreover, plant secondary metabolites are known to contribute to colour, flavour and taste of the foods (Balasa & Knorr 2011). All these parameters determine the final quality of tomato fruit, and hence, their end use or even their acceptance by consumers. Application of abiotic stress factors has been shown to significantly impact the quality attributed of fruit (Atkinson et al. 2011; Rosa et al. 2009). However, to the best of our knowledge, there are no previous studies aimed at evaluating the effect of the application of PEF treatments on quality attributes of whole fruit and vegetables.

Therefore, the objective of this study was to evaluate the respiratory activity and physicochemical quality properties of tomato fruit as

affected by PEF treatment conditions applied to elicit an enhancement in their carotenoids content.

2. Material and methods

2.1. Reagents

Butyl hydroxytoluene (BHT) was acquired from Scharlau Chemie S.A. (Barcelona, Spain). DPPH (2,2-diphenyl-1-picrylhydrazyl) and Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) were purchased from Sigma-Aldrich (St. Louis, MO, USA).

2.2. Tomatoes

Tomato fruit *(Lycopersicum esculentum* cv. Raf*)* were acquired from a local market (Lleida, Spain) at turning stage, characterized by more than 10 % but not more than 30 % of the surface showing a definite change in color from green to red (USDA 1991). The fruit were stored at 12 ± 1 °C until they reached a light red-ripe stage, hence exhibiting red color in more than 60 % but not more than 90 % of the surface (USDA 1991). Prior to PEF processing, tomatoes were rinsed with tap water. The excess of water was carefully removed from the surface with a paper cloth.

2.3. Pulsed electric field treatments

PEF treatments were conducted in a batch mode PEF system (Physics International, San Leandro, CA, USA). The equipment delivers monopolar exponential-wave pulses from a capacitor of 0.1 µF at a frequency of 0.1 Hz. The treatment chamber consists of a parallelepiped methacrylate container (0.2 x 0.08 m) with two parallel stainless steel electrodes separated by a gap of 10 cm. Tomatoes were placed into the treatment chamber filled with tap water. Different electric field strengths (0.4, 1.2 and 2 $kV·cm^{-1}$) and number of pulses (5, 18 and 30 pulses) were applied. The specific energy input corresponding to each treatment was calculated according to Luengo, Condón-Abanto, Álvarez, & Raso (2014b) and is displayed in Table 1.

Table 1. *PEF-processing treatment conditions*

Electric field strength ($kV \cdot cm^{-1}$)	Number of pulses	Specific energy input ($kJ \cdot kg^{-1}$)
0	0	Untreated
0.4	5	0.02
0.4	18	0.06
0.4	30	0.09
1.2	5	0.14
1.2	18	0.50
1.2	30	0.83
2	5	0.38
2	18	1.38
2	30	2.31

Each treatment was repeated threefold and each replicate comprised two tomato fruit. Untreated and PEF-treated tomatoes were immediately stored at 4ºC for 24 h, as previously described by Vallverdú-Queralt et al. (2013). Respiratory activity and physicochemical properties of tomatoes were then measured. Afterwards, tomatoes were ground for 20 seconds in a blender (Solac Professional Mixter BV5722, Spain), immediately freeze-dried and stored at −40 °C prior to carotenoids analysis.

2.4. Extraction and analysis of carotenoid compounds

2.4.1. Extraction

Carotenoids were extracted following the methodology proposed by Odriozola-Serrano et al., (2007) with slight modifications. Freeze-dried tomato samples (0.2 g) were weighed and mixed with 20 mL of 1 % (w/v) of butylated hydroxytoluene (BHT) in ethanol:hexane (4:3 v/v). The mixture was homogenized at 6 x*g* for 15 min at 4 ºC in a Beckman Coulter centrifuge (Avanti J-26 XP, California, United States). Then, 3 mL

of distilled water were added and the mixture was shaken and kept at room temperature to allow phase separation. The organic phase was collected and used to determine total carotenoids and lycopene contents as well as lipophilic antioxidant capacity. All the extractions were repeated twice. All procedures were performed in dim lighting in order to prevent carotenoids photodegradation.

2.4.2. Determination of total carotenoids

Total carotenoids content (TCC) was determined spectrophotometrically (CECIL CE 2021; Cecil Instruments Ltd., Cambridge, UK) following the methodology proposed by Talcott & Howard (1999). The absorbance of the organic phase was measured in triplicate at 470 nm versus a blank of hexane. TCC was calculated using the following equation (1):

$$Total\ carotenoids\ content\ (mg \cdot kg^{-1}) = \frac{A_{470}\ x\ V\ x\ 10^4}{A_{1cm}^{1\,\%}\ x\ G} \quad (1)$$

where A_{470} is the absorbance at 470 nm, V is the total volume of extract (mL), $A_{1cm}^{1\,\%}$ is the extinction coefficient of a mixture of carotenoids established in 2500 by Gross (1991) and G is the sample weight (g). Total carotenoids were expressed as $mg \cdot kg^{-1}$.

2.4.3. Determination of lycopene

Lycopene content (LC) was determined spectrophotometrically following the methodology proposed by Fish, Perkins-Veazie, & Collins (2002). The absorbance of the extracts was measured at 503 nm using hexane as a blank. LC was calculated according to equation 2.

$$Lycopene\ content\ (mg \cdot kg^{-1}) = \frac{A_{503}\ x\ MW\ x\ DF\ x\ 10^6}{\varepsilon\ x\ L} \quad (2)$$

where A_{503} is the absorbance at 503 nm, MW is the molecular weight of lycopene (536.9 $g \cdot mol^{-1}$), DF is the dilution factor, ε is the molar

extinction coefficient for lycopene (17.2 x 10^4 L·mol^{-1}·cm^{-1}) and L is the pathlength (1 cm). Lycopene content was expressed as mg·kg^{-1}.

2.4.4. Lipophilic antioxidant capacity

LAC was evaluated on the same extract used for TCC and LC determination using the colorimetric method reported by Vallverdú-Queralt et al. (2012) which is based on the free radical scavenging effect of 2,2-diphenyl-1-picrylhydrazyl (DPPH). Ten microliters of tomato extract were mixed with 90 µL of distilled water and 3.9 mL of $DPPH^{\cdot}$ solution. The mixture was shaken vigorously in a vortex and kept in the dark for 30 min. The absorbance was measured at 515 nm. Results were compared with a standard curve prepared with Trolox and expressed as µmol of Trolox equivalents (TE) per kilogram.

2.5. Respiratory activity

The respiratory activity of both untreated and PEF-treated tomatoes was determined using a static system. Just after PEF treatments, three tomatoes from each treatment (ca. 130 g) were individually placed in hermetic containers (0.5 L of capacity) for 24 h at 4 ºC. Changes in the composition of the headspace were measured twice using a gas analyser (490 Micro GC, Agilent Technologies, Santa Clara, USA). A 1.7 mL sample was withdrawn from the headspace atmosphere through an adhesive rubber septum with a syringe. Portions of 0.25 and 0.33 mL were injected for O_2 and CO_2 determination, respectively. The O_2 content was analysed with a CP-Molsieve 5Å column (10 m x 0.32 mm, df = 30 µm) at 60 ºC and 100 kPa. For quantification of CO_2, ethylene (C_2H_4) and acetaldehyde (C_2H_4O), a Pora-PLOT Q column (10 m x 0.32 mm, df = 10 µm) at 70 ºC and 200 kPa, was used. Both columns were equipped with a thermal conductivity detector. Respiration rate was calculated as mg of consumed O_2 (Ro_2) and produced CO_2 (Rco_2)·kg^{-1} h^{-1} according to Fonseca, Oliveira, & Brecht (2002). In addition, the production of ethylene (µg·h^{-1}·kg^{-1}) and acetaldehyde (ng·h^{-1}·kg^{-1}) were determined.

2.6. Physicochemical properties

Colour. The CIELab parameters (lightness, L^*; green-red chromaticity, a^*; and blue-yellow chromaticity, b^*) were utilized to characterise the external colour of three tomato fruit from each PEF-treatment using a Minolta colorimeter (Minolta CR-400, Konica Minolta Sensing, Inc., Osaka, Japan). The apparatus was set up for a D65 illuminant and 10º observer angle. A white standard plate (Y = 94.00, x = 0.3158, y = 0.3322) was used for calibration. The colour was assessed by measuring the lightness (L^*) and the a^*/b^* ratio.

Firmness. Whole tomato firmness was determined in three fruit with a TA-XT2 texture analyser (Stable Micro Systems Ltd., Surrey, England), with a 4-mm-diameter steel probe at a shearing speed of 5 $mm \cdot s^{-1}$. Results were expressed in Newtons (N).

pH. pH was determined using a Crison 2001 pH-meter (Crison Instruments S.A., Alella, Barcelona, Spain) at 25 ºC.

Soluble solids. Total soluble solids content (TSS) was determined by measuring the refraction index with an Atago RX-1000 refractometer (Atago Company Ltd., Tokyo, Japan) at 25 ºC. The results were expressed as % of soluble solids.

2.7. Statistical analysis

Statistical analyses were carried out using the JMP Pro v.12.0.1 software (SAS Institute, Cary, NC, USA). PEF treatments were run in triplicate and each parameter was analysed twice (n = 6). Results were reported as the mean ± standard deviation. Results were subjected to a factorial analysis of variance (ANOVA) followed by Tukey–Kramer *post hoc* test in order to establish statistical differences among mean values. The relationship between variables was determined using the Pearson correlation coefficient. The statistical significance level was set up at $p < 0.05$.

3. Results and discussion

3.1. Effects of PEF on carotenoids and lipophilic antioxidant capacity of tomato fruit

The application of PEF treatments significantly enhanced ($p < 0.05$) total carotenoids (TCC) and lycopene (LC) concentrations in tomato fruit (Figures 1 and 2).

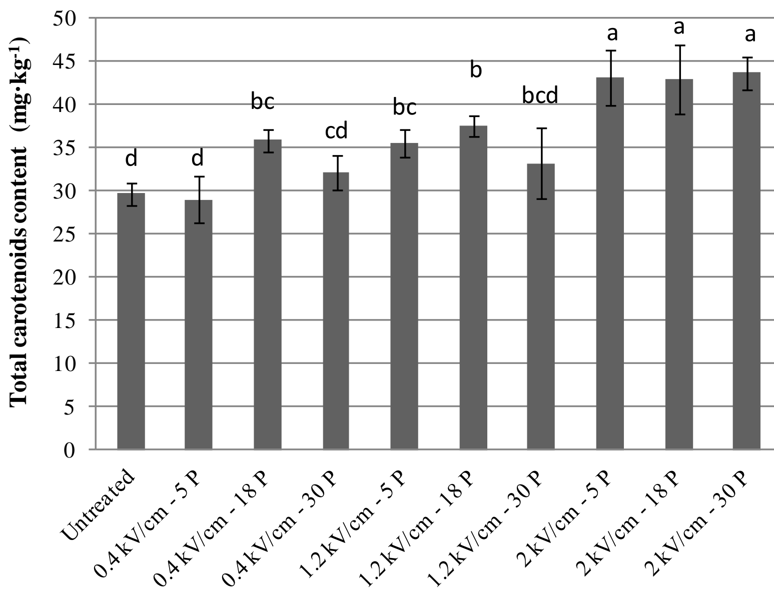

Figure 1: *Total carotenoid content (mg·kg^{-1}) of untreated and PEF-treated tomatoes.* Values are expressed as mean ± standard deviation (n = 6). Different letters mean significant differences ($p < 0.05$) on TCC between treatments.

TCC and LC were significantly ($p < 0.001$) influenced by the specific energy input applied. The electric field strength was the main treatment parameter affecting the TCC and LC of tomato, regardless the pulse number applied. Thus, TCC and LC were remarkably higher in

tomatoes subjected to 2 kV·cm^{-1}. The maximum enhancement in TCC was attained in tomatoes subjected to treatments delivering an energy input of 2.31 kJ·kg^{-1} (2 kV·cm^{-1}, 30 pulses), leading to a 50 % increase in comparison to the content in untreated fruit. However, tomatoes treated with specific energy inputs of 0.02, 0.09 and 0.83 kJ·kg^{-1} did not exhibit any significant ($p > 0.05$) change in TCC with respect to untreated fruit. Lycopene concentration increased by 53 % when tomatoes were treated at 2.31 kJ·kg^{-1}.

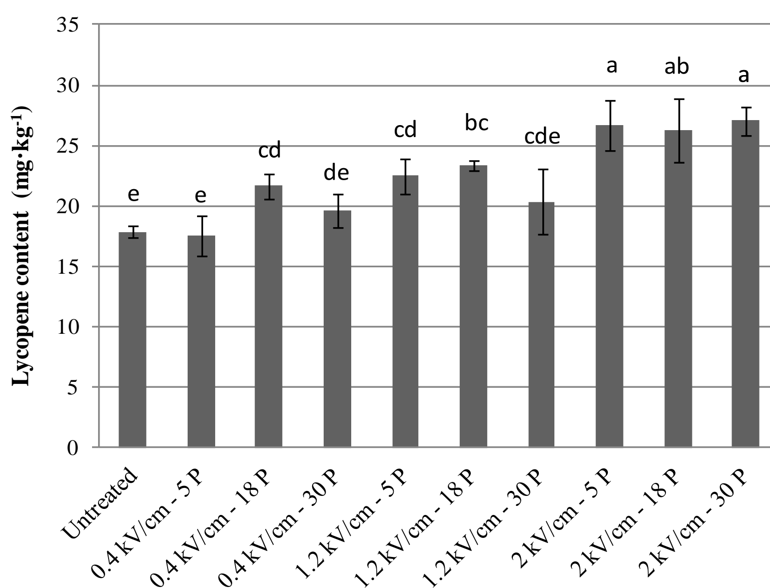

Figure 2: *Lycopene content (mg·kg^{-1}) of untreated and PEF-treated tomatoes.* Values are expressed as mean ± standard deviation (n = 6). Different letters mean significant differences ($p < 0.05$) on LC between treatments.

This trend was already observed in tomato fruit cv. Daniella by Vallverdú-Queralt et al. (2013), who attributed this phenomenon to the activation of carotenoids metabolic pathway and the increased in the extractability from the food matrix caused by a permeabilization of the cellular membrane. However, they reported less accumulation of

carotenoids and a decrease in total carotenoid concentrations after a treatment of 2 kV·cm^{-1} and 30 pulses, which is in contrast to our observations. These differences in the sensitivity of tomato fruit tissues to PEF may have a varietal component, which can be related to the different metabolic response triggered.

The increased concentration of carotenoids by PEF was also accompanied by an enhancement of the LAC of tomato fruit (Figure 3).

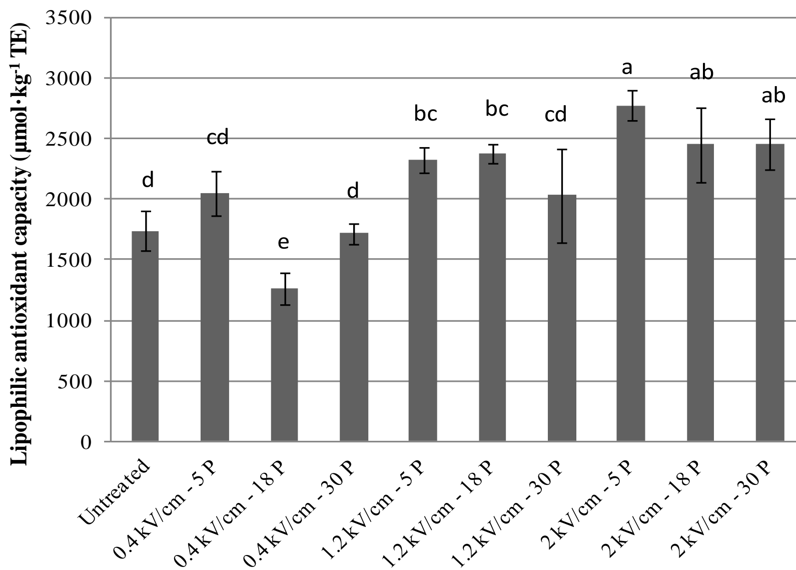

Figure 3: *Lipophilic antioxidant capacity (LAC) (µmol·kg^{-1}TE) of untreated and PEF-treated tomatoes measured by DPPH assay.* Values are expressed as mean ± standard deviation (n = 6). Different letters mean significant differences ($p < 0.05$) on LAC between treatments.

This increase in LAC values correlated well with the accumulation of TCC ($r = 0.60$, $p < 0.001$) and LC ($r = 0.62$, $p < 0.001$) (Table 4). PEF treatments produced a significant ($p < 0.05$) increase in LAC values of tomato (Figure 3), ranging from 17 % (0.02 kJ·kg^{-1}) to 60 % (0.38 kJ·kg^{-1}). The electric field strength was the main treatment parameter affecting the LAC of tomato fruit. Thus, treatments carried out at 2 kV·cm^{-1} led to

the highest increase in LAC values, regardless the number of pulses applied. The maximum enhancement in LAC was attained after applying 5 pulses at 2 kV·cm^{-1} (0.38 kJ·kg^{-1}) thus reaching values of 2.78 ± 0.08 mmol·kg^{-1} TE. This is in line with the results reported by Vallverdú et al. (2012) who also found an increase in the antioxidant capacity, ranging from 10.4 to 37.4 % in PEF-processed tomato fruit.

3.2. Effects of PEF on the respiratory activity of tomato fruit

The effect of PEF on the respiratory activity of tomato fruit is displayed in Table 2. The application of PEF treatments to tomato fruit had a determinant impact on the modification of the respiration rate, leading to increased oxygen consumption (R_{O_2}) and carbon dioxide production (R_{CO_2}). The statistical analysis revealed that both R_{O_2} and R_{CO_2} were differently influenced ($p < 0.05$) by PEF treatments differing in the specific energy input applied. R_{O_2} or R_{CO_2} were shown to be majorly affected by electric field strength, as this parameter substantially impacted the overall amount of energy delivered per mass of product.

A sharp increase in R_{O_2} was induced in tomatoes subjected to the highest specific energy inputs. A peak value in oxygen consumption after applying 0.38 kJ·kg^{-1} (2 kV·cm^{-1}, 5 pulses) (Table 2) was found, corresponding to a 156 % increase with respect to that found in untreated tomatoes. Similarly, CO_2 production markedly rose after the application of 0.38 kJ·kg^{-1}, thus reaching a maximal Rco_2 value of 7.5 ± 0.5 mg·h^{-1}·kg^{-1} of CO_2. Further increase in the amount of energy delivered resulted into a progressive reduction of the respiratory rates compared to the reported peak values (Table 2). In line with our results, Dellarosa et al., (2016) reported that PEF treatments with electric field strengths of 0.1 kV·cm^{-1} triggered the increase in R_{O2} and R_{CO2} of fresh-cut apples, whereas more intense treatments led to a sharp decrease of both R_{O2} and R_{CO2} as a consequence of a severe loss of cell viability. The increased respiratory activity in plants under abiotic stress has been observed by many authors, proving that respiration plays a special role in plant adaptation to adverse conditions (Rakhmankulova et al.

2003; Fraire-Velazquez & Emmanuel 2013). It has been reported that PEF act as an abiotic stressor, eliciting similar responses to those triggered by other stress factors. In this regard, Sabri et al. (1996) proposed a model describing the generation of ROS in plant cells induced by electropermeabilization. As suggested in their work, the disruption of cell walls, by electrical breakdown, induces a Ca^{2+} influx into cell nucleus leading to the generation of ROS. These ROS may finally act as a signal to accelerate the respiration rate (Jacobo-Velázquez et al., 2011). Moreover, various studies have reported that the modification of respiratory activity facing the presence of any stress, could be simultaneously accompanied by changes in metabolism as an adaptive strategy against unfavourable conditions (Łukaszuk, E. & Ciereszko 2012; Rakhmankulova et al. 2003; Sabbagh et al. 2014; Yuan et al. 2016). The significant correlations ($p < 0.01$) found between TCC and both R_{O2} ($r = 0.41$) and R_{CO2} ($r = 0.36$) (Table 4) indicate that the acceleration of respiratory activity of tomatoes after PEF treatments may be connected to the activation of the carotenoids biosynthetic pathway as a way to overcome oxidative stress. However, the lack of a strong correlation found could be explained by the complexity of chemical reactions occurring in natural systems as well as by the severe structural injuries caused beyond a certain energy input value, which would lead to cell death and the subsequent reduction of respiratory rates.

Ethylene production was significantly influenced by the application of PEF treatments (Table 2). Ethylene concentration was remarkably higher in tomatoes treated with the lowest electric field strength. A maximum 53 % increase was reached after the application of treatments with an energy input of 0.09 $kJ \cdot kg^{-1}$. Further increase in the intensity of PEF treatments led to a depletion in ethylene concentration. This fact could be associated to the sharp rise in CO_2 (Table 2), which has been suggested to act as a competitive inhibitor of ethylene (Soliva-Fortuny et al. 2004).

Table 2: *Effect of PEF treatment conditions on the respiratory activity of tomato*

Specific energy input (kJ·kg^{-1})	Oxygen consumption (mg·h^{-1}·kg^{-1})	Carbon dioxide production (mg·h^{-1}·kg^{-1})	Ethylene production (µg·h^{-1}·kg^{-1})	Acetaldehyde production (ng·h^{-1}·kg^{-1})
Untreated	2.09 ± 0.51 ^{c}	2.80 ± 0.16 ^{c}	1.70 ± 0.87 ^{bc}	ND ^{c}
0.02	2.29 ± 0.24 ^{c}	3.97 ± 0.26 ^{abc}	2.19 ± 0.89 ^{ab}	ND ^{c}
0.06	2.75 ± 0.58 ^{bc}	3.80 ± 0.13 ^{bc}	1.90 ± 0.90 ^{abc}	ND ^{c}
0.09	2.26 ± 0.25 ^{c}	4.09 ± 0.22 ^{abc}	2.59 ± 0.94 ^{a}	ND ^{c}
0.14	3.15 ± 0.64 ^{abc}	5.40 ± 0.34 ^{abc}	1.29 ± 0.54 ^{bcd}	ND ^{c}
0.38	5.37 ± 0.40 ^{a}	7.48 ± 0.48 ^{a}	1.31 ± 0.60 ^{bcd}	1.09 ± 0.12 ^{abc}
0.5	3.24 ± 0.79 ^{abc}	6.33 ± 0.34 ^{ab}	1.88 ± 0.73 ^{bc}	0.67 ± 0.01 ^{abc}
0.83	3.29 ± 0.47 ^{abc}	4.78 ± 0.17 ^{abc}	1.73 ± 0.26 ^{bc}	1.41 ± 0.15 ^{a}
1.38	3.10 ± 0.30 ^{ab}	4.33 ± 0.14 ^{abc}	1.21 ± 0.15 ^{cd}	0.32 ± 0.03 ^{bc}
2.31	2.11 ± 0.67 ^{c}	3.83 ± 0.26 ^{abc}	0.72 ± 0.45 ^{d}	1.10 ± 0.29 ^{ab}

Values are expressed as mean ± standard deviation (n = 6). Different letters within the same column mean statistically significant differences ($p < 0.05$) between treatments. ND: no detected.

Ethylene biosynthesis has already been reported to be involved in several processes such as ripening as well as pathogen and wounding responses, leaf senescence and biotic or abiotic stress responses (Alexander & Grierson 2002). This allows confirming the hypothesis proposed by Vallverdú-Queralt et al., (2013a) who suggested that PEF could evoke ethylene production and in turn, the activation of carotenoids biosynthesis. Moreover, the drop in ethylene concentration and the deceleration of the Ro_2 and Rco_2 (Table 2) when tomatoes were treated with the highest energy inputs suggest that these processing conditions trigger a severe loss of cell viability probably caused by irreversible pore formation in the cellular membranes.

It is worth highlighting the induction of acetaldehyde synthesis when tomatoes were subjected to specific energy inputs above 0.38 $kJ \cdot kg^{-1}$, reaching the maximum values (1.41 ± 0.15 $ng \cdot h^{-1} \cdot kg^{-1}$) in tomatoes treated with 0.83 $kJ \cdot kg^{-1}$ (30 pulses at 1.2 $kV \cdot cm^{-1}$). The presence of acetaldehyde confirms the triggering of anaerobic processes, which was possibly associated to the flooding of intracellular spaces as a result of the leaking of cellular contents. This is in line with the results obtained by Dellarosa et al., (2016) who confirmed that anaerobic fermentative metabolism took place in fresh-cut apples treated with electric field strengths ranging from 0.1 to 0.4 $kV \cdot cm^{-1}$.

3.3. Effects of PEF on physicochemical properties of tomato fruit

PEF processing had a significant effect ($p < 0.05$) on the physicochemical properties of tomato fruit (Table 3). With regard to colour, both *L** and *a*/b** ratio significantly changed 24 h after the application of PEF. Statistical analysis indicated that the electric field strength was the main PEF processing parameter affecting tomato colour ($p < 0.001$). However, a correlation between colour parameters and pulse number or specific energy input delivered could not be drawn. On the one hand, lightness *(L*)* is the main indicative parameter associated with browning of fruit and vegetables. The application of

PEF led to a decrease in lightness values, especially after delivering energy inputs beyond 0.14 $kJ·kg^{-1}$ (E ≥ 1.2 $kV·cm^{-1}$). Changes in tomato lightness could be triggered by a decompartimentalization process which allow enzymes to come into contact with their substrates as a consequence of electroporation-driven migration of cell contents (Asavasanti et al. 2010). On the other hand, high energy inputs, especially those corresponding to 2 $kV·cm^{-1}$ treatments, promoted an increase in *a*/b** values. This change was related to an increase in *a** values, which ranged from 8.3 ± 1.8 (untreated tomatoes) to 15.3 ± 0.9 (2.31 $kJ·kg^{-1}$) (data not shown). A significant ($p < 0.001$) correlation between *a*/b** ratio and both TCC (r = 0.67) and LC (r = 0.73) (Table 4) was found, which is consistent with the well-established relationship between the reddening of tomato and the accumulation of carotenoids (Arias et al. 2000).

The structural integrity of tomato tissues was strongly related to the specific energy input of the treatment (Table 3). Hence, the higher the treatment intensity the greater the softening effect. Thus, the most intense PEF treatment (2.31 $kJ·kg^{-1}$) cause a 80 % reduction in firmness values. Nevertheless, the firmness of tomato fruit was dramatically affected even for low energy treatments. This is in agreement with previous works which found that the application of electric fields of 0.1 to 5 $kV·cm^{-1}$ can induce severe tissue damage through membrane breakdown (Asavasanti et al. 2010). Additionally, the inverse correlation found between the firmness of tomato and both TCC (r = - 0.60, $p < 0.001$) and LC (r = - 0.63, $p < 0.001$) (Table 4) suggests that those conditions leading to the highest carotenoid content were also those resulting into the highest firmness loss. This could in turn favour the extraction of carotenoids from the food matrix, as reported for other vegetable tissues after the application of PEF treatments. Luengo et al., (2014a) observed an increased extraction of carotenoids from tomato peels by increasing the electric field strength up to 5 $kV·cm^{-1}$. In the same way, Zderic et al., (2013) reported an enhancement of the extraction of polyphenols from fresh tea leaves when electric field strengths ranging from 0.4 to 1.1 $kV·cm^{-1}$ were applied.

Table 3: *Physicochemical properties of untreated and PEF-treated tomato*

Specific energy input (kJ·kg^{-1})	Fruit colour		Firmness (N)	Soluble solids (%)	pH
	L^*	a^*/b^*			
Untreated	43.9 ± 2.4 ^{a}	0.40 ± 0.11 ^{d}	17.4 ± 2.1 ^{a}	4.6 ± 0.3 ^{d}	4.06 ± 0.01 ^{ef}
0.02	43.6 ± 2.1 ^{a}	0.48 ± 0.18 ^{cd}	14.2 ± 2.7 ^{bc}	4.9 ± 0.1 ^{cd}	4.10 ± 0.07 ^{de}
0.06	42.5 ± 1.9 ^{ab}	0.54 ± 0.22 ^{bcd}	14.9 ± 1.8 ^{ab}	5.2 ± 0.3 ^{abc}	4.05 ± 0.01 ^{f}
0.09	41.9 ± 1.3 ^{ab}	0.56 ± 0.15 ^{bcd}	10.7 ± 2.0 ^{c}	4.8 ± 0.1 ^{cd}	4.13 ± 0.02 ^{cd}
0.14	41.1 ± 1.8 ^{abc}	0.92 ± 0.15 ^{a}	8.7 ± 1.7 ^{cd}	4.7 ± 0.3 ^{cd}	4.11 ± 0.03 ^{cd}
0.38	39.1 ± 4.7 ^{bc}	0.90 ± 0.09 ^{a}	6.3 ± 0.4 ^{de}	4.7 ± 0.1 ^{cd}	4.70 ± 0.10 ^{a}
0.5	36.9 ± 0.9 ^{c}	0.78 ± 0.08 ^{ab}	6.1 ± 1.2 ^{de}	5.0 ± 0.7 ^{bcd}	4.18 ± 0.05 ^{b}
0.83	39.2 ± 1.4 ^{bc}	0.71 ± 0.11 ^{abc}	5.9 ± 0.7 ^{de}	5.3 ± 0.2 ^{abc}	4.20 ± 0.10 ^{bc}
1.38	38.7 ± 0.6 ^{bc}	0.92 ± 0.17 ^{a}	6.8 ± 1.6 ^{de}	5.6 ± 0.3 ^{ab}	4.10 ± 0.10 ^{cd}
2.31	40.5 ± 1.9 ^{abc}	0.88 ± 0.08 ^{a}	3.1 ± 0.7 ^{e}	5.7 ± 0.9 ^{a}	4.15 ± 0.06 ^{bc}

Values are expressed as mean ± standard deviation (n=6). Different letters within the same column represent statistically significant differences ($p < 0.05$) between treatments.

Table 4. *Pearson correlation coefficients between carotenoids content, lipophilic antioxidant activity, respiratory activity and the quality attributes of tomato fruit*

	TCC	LC	LAC	R_{O2}	R_{CO2}	Ethylene	Acetaldehyde	*L**	*a*/b**	Firmness	TSS
TCC											
LC	0,9858***										
LAC	0,6***	0,6195***									
R_{O2}	0,4084**	0,4084**	0,3799**								
R_{CO2}	0,3552**	0,3539**	0,3823**	0,6755***							
Ethylene	-0,3841**	-0,3848**	-0,2597*	0,0971	0,0527						
Acetaldehyde	0,2162	0,2406	0,2451	0,0858	-0,0196	-0,1983					
*L**	-0,4637***	-0,4599***	-0,4089	-0,179	-0,3994*	0,2417	-0,1031				
a/b**	0,6983***	0,7315***	0,532***	0,2604*	0,3597**	-0,3923**	0,1724	-0,6352***			
Firmness	-0,6041***	-0,6303***	-0,633***	-0,2779*	-0,327*	0,3211*	-0,3778**	0,55***	-0,6492***		
TSS	0,4377***	0,4183***	0,2322	0,0401	-0,1311	-0,1801	0,355**	-0,2457	0,2836*	-0,3735**	
pH	0,4592***	0,461***	0,561***	0,3679*	0,4712***	-0,156	0,1327	-0,304*	0,3747**	-0,3828**	-0,234

Significant correlation at $p < 0.05$ (*), $p < 0.01$ and (**) and $p < 0.001$(***). TCC, total carotenoids concentration; LC, lycopene concentration; LAC, lipophilic antioxidant capacity; R_{O_2}, oxygen consumption; R_{CO_2}, carbon dioxide production; *L**, lightness; TSS, total soluble solids.

PEF treatments also induced changes in total soluble solids (TSS) content of tomato. The initial TSS of untreated fruit was 4.6 ± 0.4 % and was significantly ($p < 0.05$) influenced by both the electric field strength and the number of pulses applied (Table 3). Thus, TSS values rose by 24 % and reached highest values in those fruit subjected to the most intense treatments (2.31 $kJ \cdot kg^{-1}$). It is known that soluble sugars act as metabolic and structural components of cells, however, they also take part in some processes linked to growth, development and metabolic responses of plants (Rosa et al. 2009). As soluble sugars are very sensitive to stress factors, it has already been reported an active accumulation of solutes in response to osmotic stress (Atkinson et al. 2011; Fraire-Velazquez & Emmanuel 2013). According to Toepfl et al., (2005) the membrane rupture triggered by PEF produce osmotic imbalances in cells. Therefore, the accumulation of sugars in PEF-treated tomatoes may play a role in osmoregulation as a strategy of tomato to restore the cell activity (Galindo et al. 2007; Galindo et al. 2009). In addition, the increased concentration of soluble solids could be linked with the acceleration of tomato ripening associated to the increased metabolic activity induced by PEF. Moreover, the application of these treatments may produce the disorganization of cell wall polysaccharides and molecular bonds (Cholet et al. 2014) which could lead to the release of soluble solids into the aqueous phase at membrane interfaces, modifying the TSS content.

PEF treatments also modified the natural pH of tomato. The pH of untreated tomatoes was 4.06 ± 0.01 and significantly ($p < 0.05$) increased when tomatoes were subjected to PEF treatments delivering energy inputs beyond 0.09 $kJ \cdot kg^{-1}$. The maximum pH values were found in tomatoes treated at 2 $kV \cdot cm^{-1}$ and 5 pulses. After such treatments, tomato fruit also exhibited their maximum peak on both R_{O2} and R_{CO2}. Therefore, the increased pH values could be related to higher respiration rate after PEF treatments where organic acids were used as substrate. To the best of our knowledge there are no previous studies explaining the changes in pH when PEF treatments were applied to whole fruit, even though Kader and Lindberg, (2010) reported that changes in intracellular pH acts as secondary messenger in response of

plants to different stress conditions. In addition, the modification of pH in PEF-treated tomatoes may be attributed to the electrical breakdown of cell membranes, which could become more permeable to molecules and ions that are sufficiently small to traverse membrane pores (Garner et al. 2007). However the complexity of pH signalling against stress factors makes necessary to carry out additional studies in order to clarify the specific role of pH in plant defence mechanism to PEF-induced stress.

4. Conclusions

Pulsed electric field (PEF) treatments enhanced the amount of carotenoids in tomato fruit. PEF treatments conducted at 2 $kV \cdot cm^{-1}$ and 30 pulses led to the maximum increase in total carotenoids (50 %) and lycopene (53 %) concentration. The stress-induced accumulation of carotenoids was accompanied by changes in the respiratory activity as well as in the main physicochemical properties of tomato fruit. Increased values of pH and TSS, as well as changes in the surface colour were found after applying PEF treatments. However, irreversible damage in tomato tissue promoted by PEF led to a dramatic loss of firmness, which in turn affected the appearance and overall quality of tomato fruit. Therefore, PEF could be proposed as a pre-processing treatment to produce tomato-based products with high antioxidant potential. However, the precise control of processing conditions is fundamental for the feasible application of this promising technology.

5. Abbreviations

PEF, pulsed electric fields; TSS, total soluble solids; TCC, total carotenoids content; LC, lycopene content; LAC, lipophilic antioxidant capacity; ROS, reactive oxygen species; BHT, butyl hydroxytoluene; DPPH, 2,2-diphenil-1-picrylhydrazyl; R_{O_2}, oxygen consumption; R_{CO_2}, carbon dioxide production; *L**, lightness; *a**, green-red chromaticity; *b**, blue-yellow chromaticity; TE, Trolox equivalents; ANOVA, analysis of variance; E, electric field strength.

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CHAPTER 2

Evaluation of physicochemical properties and carotenoid profile of tomato fruits during storage at different temperatures after pulsed electric fields processing

ABSTRACT

Pulsed electric fields (PEF) have been proposed to elicit an increase in the health-related compounds content of fruits. Nevertheless, the metabolic response of tomato is known to be greatly affected by postharvest storage conditions and may have a determinant impact on the quality characteristics of the product. The effects of PEF processing and post-treatment storage conditions, namely time and temperature, on both the carotenoid profile and the main physicochemical properties of tomato fruits were evaluated. The application of PEF treatments significantly improved the accumulation of carotenoids in tomato fruits. Nevertheless, the concentration of each individual carotenoid during storage was differently influenced by the storage temperature depending on the previously applied PEF treatment. The increased concentration of carotenoids was noticeably higher in tomatoes stored at 12 ºC than in those fruits stored at 4 or 20 ºC. The most intense PEF treatment (0.38 $kJ\cdot kg^{-1}$) triggered a fast accumulation of carotenoids, leading to the maximum concentrations of β-carotene (77%), γ-carotene (200%) and lutein (238%) in tomatoes stored at 12 ºC for 1 day. However, irreversible damage was caused to tomato tissues, thus leading to undesired effects on the fruit quality. On the other hand, the mildest PEF treatment (0.02 $kJ\cdot kg^{-1}$) promoted the greatest accumulations of total carotenoids (58%) and lycopene (150%) in tomatoes stored during 5 days at 12 ºC without compromising the fresh-like quality of tomato fruits. The results obtained provide valuable information for the future application of PEF in the development of tomato derivative products with increased health-related properties.

1. Introduction

Consumption of raw tomatoes and tomato-based products is nowadays strongly associated with a reduced incidence of certain types of cancer, cardiovascular diseases and atherosclerosis (Hedges & Lister, 2005). These health-promoting properties have been attributed to the presence of high amounts of phytochemicals, including carotenoids, which act as antioxidants in detoxifying free radicals (Ilahy, Hdider, Lenucci, Tlili, & Dalessandro, 2011; Vallverdú-Queralt, Oms-Oliu, et al., 2013).

The increased demand of healthy foods provides an opportunity to develop new technologies that allow obtaining products with enhanced functional properties. Pulsed electric fields (PEF) treatments have attracted large interest due to its potential to offer useful applications in the food industry. Inactivation of microorganism and enzymes (Elez-Martínez, Sobrino-López, Soliva-Fortuny, & Martín-Belloso, 2012; Martín-Belloso & Elez-Martínez, 2005), extraction of intracellular compounds (Luengo, Condón-Abanto, Álvarez, & Raso, 2014; Vorobiev & Lebovka, 2006), preservation of certain food components (Odriozola-Serrano, Soliva-Fortuny, & Martín-Belloso, 2009), among others, have been investigated. In addition, some authors have proposed the application of PEF at moderate intensity as an abiotic elicitor capable of inducing an increase in the antioxidant content of plant tissues (Soliva-Fortuny, Vendrell-Pacheco, Martín-Belloso, & Elez-Martínez, 2017; Vallverdú-Queralt, Oms-Oliu, et al., 2013; Ye, Huang, Chen, & Zhong, 2004). The biosynthesis of secondary metabolites in metabolically active fruits may be stimulated through a broad range of factors. These signals are thought to promote the formation of reactive oxygen species (ROS), which would in turn stimulate the biosynthetic pathway of secondary metabolites in order to overcome stressful conditions (Balasa & Knorr, 2011). Preliminary studies have demonstrated the feasibility of applying PEF treatments to trigger the accumulation of some phytochemicals. Vallverdú-Queralt et al. (2013) observed a maximum increase in total carotenoids content (1.38-fold increase) when tomato fruits were stored at 4 ºC for 24 h after the application of

5 pulses at 1.2 kV·cm^{-1}. In addition, Soliva-Fortuny et al. (2017) noticed that the application of PEF treatments produce significant greater concentration of phenolic compounds in apples. However, associated with the stress-adaptive response to PEF, several changes in quality attributes could be affected. It is known that PEF could strongly affect the structural integrity of cell walls, and hence the firmness of fruits and vegetables (Lebovka, Praporscic, & Vorobiev, 2004; Shayanfar, Chauhan, Toepfl, & Heinz, 2013). This fact could lead to undesirable effects on the final quality of tomato fruits.

Stress response in plant tissues is thought to be affected by internal and external factors (Hodges & Toivonen, 2008). On the one hand, the internal factors represent metabolic responses and may include morphological, physiological and biochemical defence mechanisms. On the other hand, the external factors represent environmental conditions which may intensify or inhibit the manifestation of the internal factors. The storage conditions are including among these last factors. It is well established that proper control of postharvest storage conditions, mainly temperature, is critical to maintain quality and to extend the self-life of tomatoes (Lana, Tijskens, & Van Kooten, 2005). In this regard, previous studies have been aimed at evaluating the influence of storage temperature on quality and metabolic behaviour of intact tomato fruits, including the biosynthetic pathway of carotenoids (Javanmardi & Kubota, 2006; Vinha, Barreira, Castro, Costa, & Oliveira, 2013). However, there is a lack of knowledge regarding the effects of the storage conditions on the elicited biosynthesis of carotenoids in tomato fruits subjected to different PEF treatment conditions. Therefore, the objective of this work was to study the accumulation of carotenoids in tomato fruits as well as the main modifications in their physicochemical properties as affected by PEF treatment intensity and the storage conditions, namely time and temperature.

2. Material and methods

2.1. Reagents

Butylated hydroxytoluene (BHT) was acquired from Scharlau Chemie S.A. (Barcelona, Spain). Magnesium hydroxide carbonate was purchased from Sigma-Aldrich (St. Louis, MO, USA). Lycopene, γ-carotene, δ-carotene, β-carotene, lutein, phytofluene and phytoene standards were obtained from Carote-Nature (Ostermundigen, Switzerland).

2.2. Tomato fruits

Tomato fruits (*Lycopersicum esculentum* cv. Raf) were obtained from a local supplier in Lleida (Spain). The fruits were acquired at turning stage, which means that more than a 10% but not more than a 30% of the surface showed a definite change in colour from green to red (USDA, 1991). Tomatoes were then stored at 12 ± 1 ºC until they reached a light-red stage (60 - 90% of the surface showing red colour) (USDA, 1991). Prior to PEF treatments, tomatoes were rinsed with tap water and dried carefully with paper cloth to remove adhering dirt on their surface.

2.3. Pulsed electric field treatments

PEF treatments were carried out with a device manufactured by Physics International (San Leandro, CA, USA). The apparatus delivers monopolar exponential-wave pulses from a capacitor of 0.1 μF with a frequency of 0.1 Hz. Treatments were conducted in batch mode. The treatment chamber was a parallelepiped methacrylate container (200 x 80 mm) with two parallel stainless steel electrodes separated by a gap of 10 cm. Tomato fruits were subjected to either 5 pulses at 0.4 or 5 pulses at 2 $kV{\cdot}cm^{-1}$, resulting in specific energy inputs of 0.02 $kJ{\cdot}kg^{-1}$ and 0.38 $kJ{\cdot}kg^{-1}$, respectively. These PEF conditions were selected according to the results obtained in preliminary experiments. Each treatment was repeated fourfold and each replicate comprised two tomato fruits.

2.4. Storage conditions

Immediately after PEF processing, tomatoes were stored in darkness at 4, 12 or 20 ºC for different storage times (0, 1, 3 and 5 days). Untreated tomatoes were used as a reference. At specific storage times, both untreated and PEF-treated tomatoes were withdrawn from the storage chambers. Physicochemical properties (colour, texture, pH and total soluble solids) from each tomato fruit were then determined. Afterwards, tomatoes from each treatment batch were ground (Solac Professional Mixter BV5722, Spain). Homogeneous samples were then freeze-dried and stored at −40 °C until carotenoids extraction. The detailed methodologies to determine each parameter are described hereafter.

2.5. Carotenoids

2.5.1. Extraction of carotenoids

Carotenoid extraction and quantification was carried out following the methodology proposed by Rodríguez-Roque, et al. (2013) with minor modifications. One gram of freeze-dried tomato sample was mixed with 0.1% (w/w) magnesium hydroxide carbonate and 10 mL of 0.05% (w/v) BHT in ethanol:hexane (4:3 v/v). The mixture was homogenized using an Ultraturrax T-25 Basic (IKA®-Werke GmbH & Co., Staufen, Germany) for 2 min in an ice-bath. Afterwards, it was filtered under vacuum through grade 1 Whatman paper. The residue was re-extracted once with 10 mL of ethanol:hexane (4:3 v/v) for 2 min with an Ultraturrax. Then, the mixture was again filtered and the residue was washed twice with 5 mL of ethanol and once with 5 mL of hexane. All the filtrates were combined in an amber round-bottom flask and rotoevaporated (rotovapor R-3000, BUCH, Switzerland) at 45 ºC for 15 min to dryness. The residue was then saponified under a N_2 atmosphere by adding 10 mL of methanolic KOH 0.5 M + 0.1% BHT (v/w) and 10 mL of diethyl ether for 30 min with continuous agitation. Afterwards, the extract was placed in an amber decanting funnel and washed twice with 25 mL of 10% NaCl solution and thrice with 25 mL of distilled water. The aqueous phase was discarded each time. The organic phase was collected and

rotoevaporated at 45 °C for 20 min to dryness. The residue was dissolved with 4 mL of diethyl ether and placed in an amber glass vial. Finally, the solvent was evaporated under a N_2 flow and stored at −40 °C until analysis. Before injection into the HPLC system, the carotenoid extract was reconstituted with 1 mL of methylene chloride and filtered through a 0.45 µm filter. All the extractions were conducted in duplicate.

2.5.2. Analysis of carotenoids

Carotenoids were quantified by high-performance liquid chromatography (HPLC) following the methodology reported by Odriozola-Serrano et al. (2009). The HPLC system was equipped with a 600 controller and a diode array detector 2996 (Waters Corp.) set to scan from 240 to 550 nm. Separations were performed on a reverse-phase C18 Spherisorb® ODS2 (5 µm) stainless steel column (4.6 mm x 250 mm) at room temperature with a flow rate of 0.7 $mL \cdot min^{-1}$. The gradient was as follows: 0 – 10 min, acetonitrile (85%), methanol (10%), methylene chloride (3%) and hexane (2%); 10 – 40 min, acetonitrile (45%), methanol (10%), methylene chloride (23%) and hexane (22%); and 40 -60 min, acetonitrile (85%), methanol (10%), methylene chloride (3%) and hexane (2%). Carotenoids were tentatively identified on the basis of the retention times and absorption spectrum characteristics, as previously described by Khachik et al. (1992). Their quantification was carried out by comparison with external standards of lycopene, γ-carotene, δ-carotene, β-carotene, lutein, phytofluene and phytoene. The content for each carotenoid compound was expressed as $\mu g \cdot kg^{-1}$ on a fresh weight basis (fw). Total carotenoid concentration was calculated as the sum of individual compounds and also expressed as $\mu g \cdot kg^{-1}$ (fw).

2.6. Physicochemical properties

2.6.1. Colour

Tomato surface colour was measured using a Minolta colorimeter (Minolta CR-400, Konica Minolta Sensing, Inc., Osaka, Japan). The

equipment was set up for a D65 illuminant and a 10º observer angle. A white standard plate (*Y* = 94.00, *x* = 0.3158, *y* = 0.3322) was used for calibration. The values of $L*$ (lightness), $a*$ (green to red colour), and $b*$ (blue to yellow colour) were determined. Tomato colour was assessed by measuring the lightness ($L*$) and hue angle (hº), which was calculated using equation 1.

$$hº = tan^{-1} \frac{b^*}{a^*} \quad (1)$$

2.6.2. Firmness

Firmness was evaluated by measuring the maximum penetration force for a 4-mm-diameter steel probe using TA-XT2 texture analyser (Stable Micro Systems Ltd., Surrey, England). The fruits were placed so that the plunger penetrated the pericarp approximately 1 cm away from their geometric centre to a depth of 10 mm at a rate of 5 $mm \cdot s^{-1}$. Results were expressed in Newtons (N).

2.6.3. Total soluble solids

Total soluble solids (TSS) were determined by measuring the refraction index with an Atago RX-1000 refractometer (Atago Company Ltd., Tokyo, Japan) at 25 ºC. Results were expressed as ºBrix.

2.6.4. pH

A Crison 2001 pH-meter (Crison Instruments S.A., Alella, Barcelona, Spain) was used to measure the pH values of the fruit flesh at 25 ºC.

2.7. Statistical analysis

Statistical analysis was performed using the JMP Pro v. 12.0.1 statistic software (SAS Institute, Cary, NC, USA). Results were expressed as mean ± standard deviation (n = 8). Differences between mean values were evaluated by analysis of variance (ANOVA). Subsequently, a

Tukey-Kramer post-hoc test was carried out in order to establish differences with a significance level of 0.05.

3. Results and discussion

3.1. Physicochemical properties

3.1.1. Colour

The effects of PEF treatments and post-treatment storage conditions on colour parameters (*L** and *hº*) of tomato fruits are shown in Figure 1 and 2, respectively. Untreated tomatoes exhibited initial *L** and *hº* values of 45 ± 1 and 80 ± 3, respectively. Immediately after PEF processing, tomatoes treated with an energy input of 0.38 $kJ \cdot kg^{-1}$ showed a significant ($p < 0.05$) decrease in *L** values as well as a rise in *hº* values. In contrast, the application of milder treatments (0.02 $kJ \cdot kg^{-1}$) did not produce any significant instant change in colour parameters. Changes in *L** values usually denote colour darkening of tomatoes, whereas *hº* has been usually associated to modifications in their characteristic red colour, as a consequence of carotenoid biosynthesis. Nevertheless, as colour was measured immediately after the application of PEF, colour changes are likely to be due to structural changes caused in the fruit tissue. The most intense treatment conditions probably induced the decompartmentalization of oxidative enzymes, thus allowing them to come into contact with substrates of oxidation and browning processes (Asavasanti, Ersus, Ristenpart, Stroeve, & Barrett, 2010).

Colour changes over storage of tomato fruits were differently affected by the PEF treatment intensity and the storage temperature (Figure 1 and 2). Changes in colour parameters (*L** and *hº)* in both untreated and PEF-treated tomatoes were found to be significantly influenced by the storage temperature. In this regard, tomatoes stored at 4 ºC exhibited lower rate of colour development in comparison to those kept at 12 or 20 ºC. Other authors have already observed that colour development in intact tomatoes are strongly related to the storage temperature (Žnidarčič & Požrl, 2006). As reported in their work, chilled storage

reduces enzymatic activities and hence colour development of tomato, which is in accordance with our results. Nevertheless, to the best of our knowledge, no previous works have assessed the influence of storage temperature on the colour of tomato fruits as affected by PEF.

*L** values significantly ($p < 0.05$) decreased in untreated tomatoes, reaching values of 41.9 ± 0.7 after 5 days of storage at 20 ºC. The *L** values of tomato fruits were not significantly ($p > 0.05$) influenced by the application of PEF treatments with 0.02 $kJ \cdot kg^{-1}$ in comparison to untreated tomatoes, regardless the assessed storage temperature. In contrast, tomato fruits subjected to PEF treatments delivering an energy input of 0.38 $kJ \cdot kg^{-1}$ exhibited significantly ($p < 0.05$) lower *L** values (from 42 ± 2 to 40.69 ± 0.08) throughout the storage period in comparison to those found in untreated tomatoes (from 45.6 ± 1.1 to 41.0 ± 1.3). The *hº* values of untreated and PEF-treated tomatoes significantly ($p < 0.05$) decreased throughout the storage, especially in those fruits stored at 20 ºC. PEF-treated tomatoes did not exhibit significant differences ($p > 0.05$) in *hº* values in comparison to untreated fruits, with the exception of those treated with 0.38 $kJ \cdot kg^{-1}$ and stored at 20 ºC. Under these treatment and storage conditions, *hº* values were significantly higher (from 85 ± 3 to 72 ± 7) than those observed in untreated fruits (from 80 ± 3 to 50.1 ± 1.3), thus denoting a delay in the development of red colour under those conditions. This delay in the reddening of tomato tissues could be related to the concomitant effect of intense PEF treatments and abusive storage temperatures. In this regard, the extent of tissue electroporation and the associated loss of cell viability has been reported to be tightly related with the intensity of the PEF treatments (Martín Belloso & Soliva Fortuny, 2011). Our results suggest that storage temperature below 12 ºC could compensate the deleterious effect of intense PEF treatments on cell viability, thus allowing the normal colour development in tomato fruits.

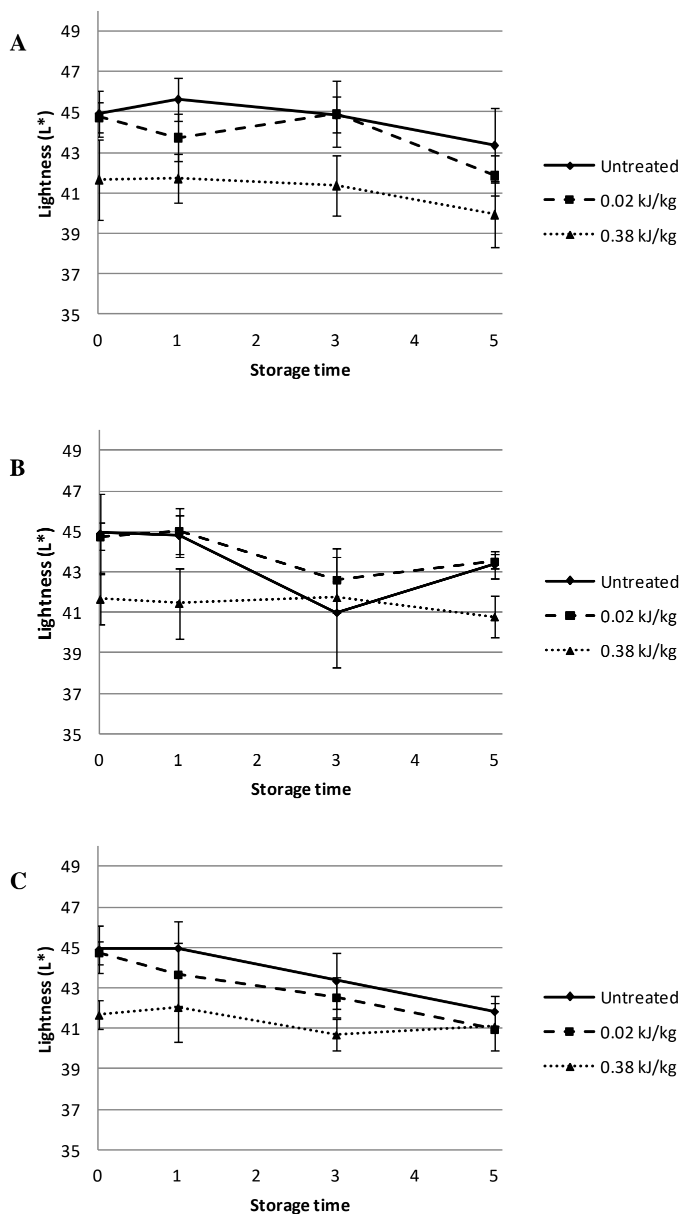

Figure 1. *Effects of PEF treatment intensity and storage time on the lightness of tomato fruits at 4 °C (A), 12 ºC (B) and 20 °C (C).* Values are means ± standard errors (n = 8).

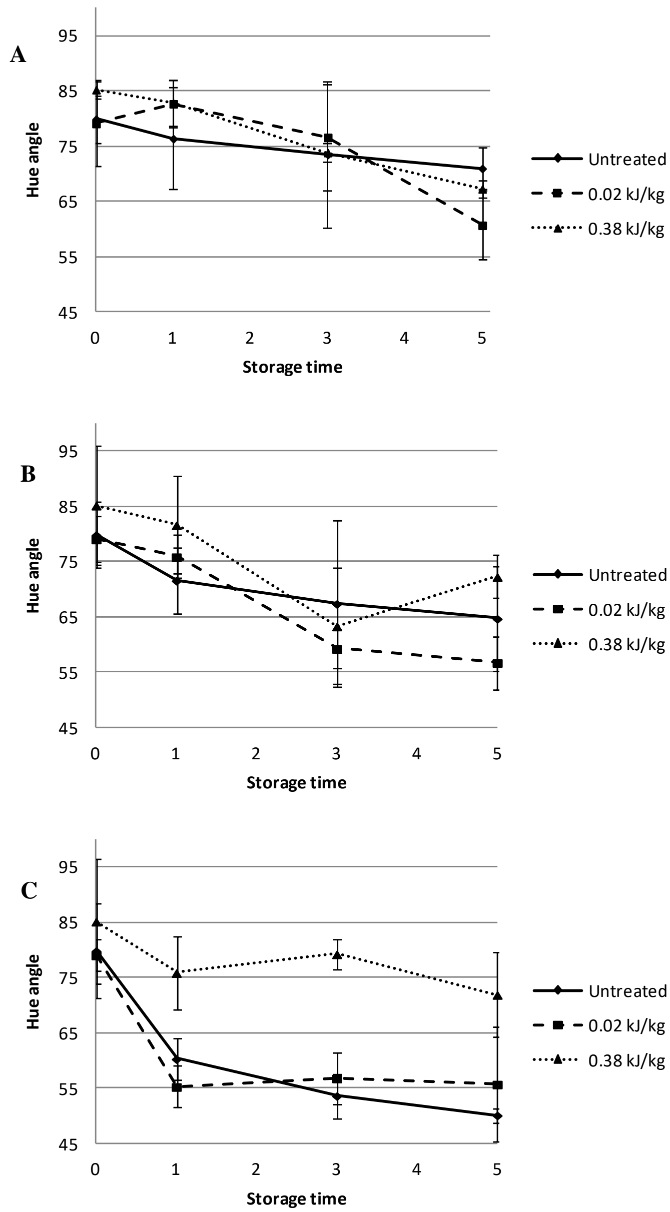

Figure 2. *Effects of PEF treatment intensity and storage time on the hue angle of tomato fruits stored at 4 °C (A), 12 ºC (B) and 20 °C (C).* Data represents the mean and standard deviation (n = 8).

3.1.2. Firmness

Changes in firmness of tomato fruits as affected by PEF treatments and storage conditions are shown in Figure 3. Tomato fruits subjected to the most intense PEF treatment (0.38 $kJ \cdot kg^{-1}$) instantly lost a 44% of their initial firmness. Conversely, the application of treatments delivering an energy input of 0.02 $kJ \cdot kg^{-1}$ did not significantly ($p > 0.05$) affect tomato firmness. The impact of PEF treatments on the texture and structure of plant tissues is well described in literature (Vorobiev & Lebovka, 2009). Structural integrity is strongly related to the intensity of PEF treatments. Thus, treatments carried out at 0.4 $kV \cdot cm^{-1}$ did not appear to produce significant modifications in tomato at the cell membrane level. In contrast, treatments conducted at 2 $kV \cdot cm^{-1}$ apparently caused major damage to cell membranes, thus leading to evident signs of softening. These results are in agreement with literature data, which report critical field strengths exceeding the threshold of irreversible electroporation of membranes in plant tissues within the range of 1 - 2 $kV \cdot cm^{-1}$ (Soliva-Fortuny, Balasa, Knorr, & Martín-Belloso, 2009).

Firmness was scarcely affected by storage under low temperature conditions. Thus, for both untreated and PEF-treated tomatoes, major firmness changes did not occur over storage at 4 and 12 ºC. However, storage at 20 ºC led to significant ($p < 0.05$) tissue softening over the storage period, regardless the applied PEF treatment. During tomato softening, pectins typically undergo solubilisation and depolymerisation, which contribute to wall disintegration (Požrl, Žnidarčič, Kopjar, Hribar, & Simčič, 2010). In line with our results, this process has been shown to be strongly inhibited over chill storage (Tadesse, Ibrahim, & Abtew, 2015).

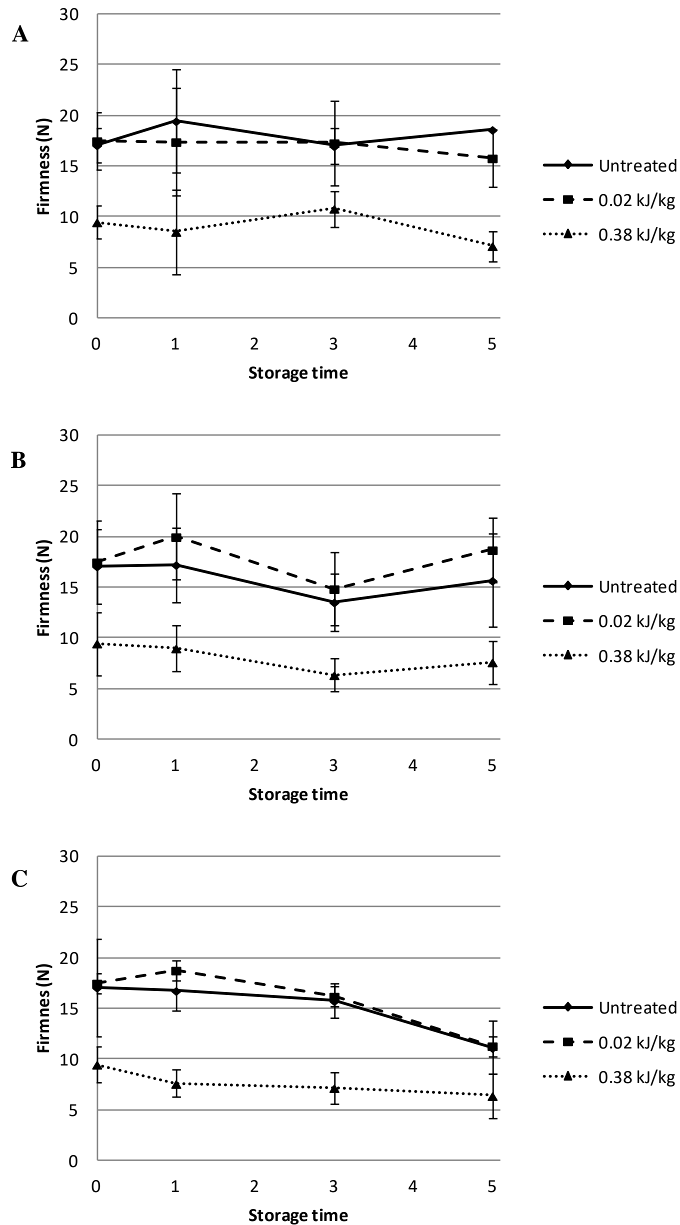

Figure 3. *Firmness changes of untreated and PEF treated tomatoes stored at 4 ºC (A), 12 ºC (B) or 20 ºC (C).* Values are means ± standard errors (n = 8).

3.1.3. Total soluble solids (TSS) and pH

The effects of PEF treatments and post-treatment storage conditions on TSS and pH of tomato fruits are shown in Table 1. Untreated tomatoes exhibited initial TSS and pH values of 5.05 ± 0.48 and 3.935 ± 0.005, respectively. TSS content was not found to be immediately affected after PEF processing. In contrast, the application of PEF treatments with energy inputs of 0.02 and 0.38 $kJ \cdot kg^{-1}$ caused an instant increase ($p < 0.05$) in pH of tomatoes in comparison to the values observed in untreated fruits. Changes in intracellular pH have been previously associated to the role of cytosolic ions, such as Ca^{2+}, which act as secondary messengers in the response of plants under stress conditions (Kader & Lindberg, 2010). Rakhmankulova and others (2003) reported that respiration plays a special role in plant adaptation to stressful conditions as the crossroads of total metabolism. Hence, the increase in pH values is likely to be related to the acceleration of tomato respiration, where organic acids were used as substrate.

As storage progressed, TSS and pH in tomato fruits differed depending on the energy input delivered and the post-treatment storage temperature (Table 1). Untreated tomatoes exhibited a continuous rise in TSS and pH values during storage, which may be associated to the accumulation of sugars, such as glucose and fructose, and a decrease in organic acids, respectively (Anthon, Lestrange, & Barrett, 2011; Fanciullino, Bidel, & Urban, 2014). PEF treatments with an energy input of 0.02 $kJ \cdot kg^{-1}$ did not exert any change in pH values of tomato during storage in comparison to untreated fruits. In contrast, treatments delivering higher energy input (0.38 $kJ \cdot kg^{-1}$) led to a significant increase in pH values, especially in tomatoes stored at 20 ºC. At such storage temperature, the rise in pH values was much greater and faster than that observed in tomato fruits stored at chilling temperatures (4 and 12ºC), thus reaching pH values of 4.150 ± 0.032 on the third storage day.

Table 1. *pH and total soluble solids content (ºBrix) of tomato as affected by PEF processing and post-treatment storage conditions.*

Parameter	Storage temperature	Treatments (kJ/kg)	Days of storage: 0	1	3	5
pH	4 ºC	Untreated	3,935 ± 0,005 Bc	4,035 ± 0,027 CDab	4,015 ± 0,005 Db	4,050 ± 0,021 CDa
		0,02	4,030 ± 0,043 Aab	4,065 ± 0,016 BCa	4,010 ± 0,011 Db	4,050 ± 0,021 CDa
		0,38	4,030 ± 0,021 Ac	4,035 ± 0,005 CDc	4,075 ± 0,005 Bb	4,100 ± 0 Aa
	12 ºC	Untreated	3,935 ± 0,005 Bc	3,975 ± 0,016 Fb	4,060 ± 0,011 BCa	4,065 ± 0,027 BCa
		0,02	4,030 ± 0,043 Aa	3,990 ± 0,011 EFb	4,045 ± 0,005 Ca	4,030 ± 0,032 DEa
		0,38	4,030 ± 0,021 Ac	4,075 ± 0,027 Bb	4,130 ± 0,032 Aa	4,080 ± 0 ABb
	20 ºC	Untreated	3,935 ± 0,005 Bc	4,030 ± 0,021 CDab	4,015 ± 0,005 Db	4,040 ± 0,011 CDa
		0,02	4,030 ± 0,043 Aa	4,020 ± 0,032 DEa	4,035 ± 0,016 CDa	4,010 ± 0,011 Ea
		0,38	4,030 ± 0,021 Ac	4,120 ± 0,032 Aab	4,150 ± 0,032 Aa	4,100 ± 0 Ab
TSS (ºBrix)	4 ºC	Untreated	5,05 ± 0,48 Ab	6,05 ± 0,69 ABa	4,75 ± 0,37 Db	4,85 ± 0,48 CDEb
		0,02	4,90 ± 0,32 Ab	5,35 ± 0,05 BCDa	5,15 ± 0,37 CDab	5,15 ± 0,37 BCDEab
		0,38	4,75 ± 0,16 Ac	6,45 ± 0,37 Aa	5,80 ± 0 Bb	4,95 ± 0,48 DEc
	12 ºC	Untreated	5,05 ± 0,48 Aa	5,00 ± 0 Da	5,25 ± 0,59 CDa	5,20 ± 0,11 BCDa
		0,02	4,90 ± 0,32 Ab	5,20 ± 0 CDa	5,35 ± 0,05 BCa	5,40 ± 0 ABCa
		0,38	4,75 ± 0,16 Ac	5,20 ± 0,43 CDb	6,70 ± 0,11 Aa	4,75 ± 0,05 Ec
	20 ºC	Untreated	5,05 ± 0,48 Aa	5,30 ± 1,07 BCDa	5,05 ± 0,48 CDa	5,00 ± 0 CDEa
		0,02	4,90 ± 0,32 Ab	5,95 ± 0,05 ABCa	5,00 ± 0,11 CDb	5,80 ± 0,11 Aa
		0,38	4,75 ± 0,16 Ab	5,55 ± 0,16 BCDa	4,20 ± 0 Ec	5,50 ± 0,11 ABa

Data represent mean values ± standard deviation (n = 8). Values with different capital letters within the same column are significantly different ($p < 0.05$), while means with different lowercase letters within the same row are significantly different ($p < 0.05$).

On the other hand, PEF treatments with an energy input of 0.38 kJ·kg^{-1} also led to a maximum 37% increase in TSS in tomatoes stored at 12 ºC for 3 days. These observations could be related to the acceleration of tomato metabolism induced by PEF, which may lead to a faster accumulation of sugars and a sharper loss of organic acids. All these processes have a biochemical nature, and hence are temperature-dependent (Lana et al., 2005). Consequently, the variation of TSS and pH values was more pronounced when tomatoes were stored at higher temperatures. Therefore, the post-treatment storage temperature would condition the accumulation of sugars and degradation of organic acids in PEF-treated tomatoes.

3.2. Carotenoid profile

The concentration of individual and total carotenoids in tomato fruits was affected by storage time and temperature, as well as by the interaction of these factors with the PEF processing conditions (Table 2). The specific energy input delivered influenced the initial concentration of carotenoids in PEF-treated tomatoes. Total and individual carotenoid concentrations were significantly enhanced by 23-171% in just-treated tomato fruits subjected to 2 kV·cm^{-1}. However, less intense PEF treatments (0.4 kV·cm^{-1}, 5 pulses) did not produce any significant instant change ($p > 0.05$) in the content of each individual compound. The increased concentration of carotenoids immediately after PEF processing at the highest electric field strength (2 kV·cm^{-1}, 5 pulses) is likely to be due to the electroporation effect of PEF. The loss of structural integrity, which was reflected in a dramatically loss of firmness in tomato fruits (Figure 2), may favour the release and extraction of carotenoids located inside the cells. This is consistent with the results reported by Luengo, Condón-Abanto, Álvarez, & Raso, (2014) who concluded that extraction yield of carotenoids from *Chlorella vulgaris* cells increased immediately after PEF processing by increasing the electric field strength applied.

As storage progressed, the concentration of carotenoids in tomatoes was differently affected depending on the PEF energy input delivered

and the post-treatment storage temperature (Table 2). A peak total carotenoid concentration of 14912 ± 845 μg·kg^{-1} was reached in those fruits subjected to an overall energy input of 0.02 kJ·kg^{-1} and subsequently stored during 5 days at 12 ºC. On the other hand, the application of the most intense PEF treatment (0.38 kJ·kg^{-1}) yielded a similar increase in the total carotenoids content, up to 13169 ± 747 μg·kg^{-1}, only 24 h after the treatment application, to subsequently decrease, when tomatoes were stored at 12 ºC. These results indicate that the application of higher intense PEF treatments led to a faster accumulation of carotenoids in tomato fruits during storage, which seems to point out that the changes in tomato metabolism can vary depending on the intensity of the stress imposed. This is in accordance with those results previously reported by Vallverdú-Queralt et al., (2012), who observed a fast metabolic response in tomato fruits when electric field strength increased.

With regard to individual carotenoid compounds, their concentration exhibited maximum values in PEF-treated tomatoes stored at 12 ºC. This indicates that the activation of the biosynthesis of carotenoids by PEF was temperature dependent. The highest enhancement in phytoene (53%) and phytofluene (60%) was reached in tomatoes subjected to PEF treatments with an energy input of 0.38 kJ·kg^{-1} and subsequently stored at 12 ºC during 3 days. Preliminary studies suggest that the production of phytoene and phytofluene could be explained by the activation of genes encoding enzymes, such as phytoene synthase, which is considered the first committed step in the carotenoids biosynthetic pathway (Vallverdú-Queralt, Oms-Oliu, et al., 2013). In a similar way, lycopene content increased by 70% in tomato fruits treated with the highest energy input (0.38 kJ·kg^{-1}) and subsequently stored at 12 ºC for 1 days. However, milder PEF treatments (0.02 kJ·kg^{-1}) led to a slower and higher increase in lycopene concentration, reaching its maximum enhancement (150%) in tomatoes stored at 12 ºC for 5 days. In accordance with the results reported by Vallverdú-Queralt, Oms-Oliu, et al. (2013), these results indicate that PEF treatments may also induce the activation of the carotenoid isomerase enzyme (CRTISO), thus resulting in greater accumulation of lycopene.

Table 2.A. *Carotenoid profile of tomato as affected by PEF processing and post-treatment storage conditions (4ºC)*

		Temperature (4 ºC)							
Time of storage	Treatments (kJ/kg)	Carotenoids concentration (µg/kg of tomato)							
		Phytoene	Phytofluene	Lycopene	δ-carotene	Lutein	γ-carotene	β-carotene	Total carotenoids
Just treated	Untreated	566 ± 108 B b	505 ± 148 B b	1984 ± 471 B b	112 ± 16 A a	255 ± 117 B b	146 ± 28 B a	3686 ± 653 B b	7254 ± 785 B b
	0.02	575 ± 94 B c	558 ± 110 B c	2305 ± 1036 B b	114 ± 21 A b	328 ± 48 B c	160 ± 41 AB a	3822 ± 753 B b	7749 ± 935 B c
	0.38	711 ± 198 A b	807 ± 259 A a	3070 ± 630 A a	112 ± 21 A b	693 ± 83 A a	180 ± 42 A ab	4576 ± 675 A b	10038 ± 989 A bc
1 day	Untreated	718 ± 72 B a	684 ± 74 B a	2584 ± 585 B a	111 ± 12 B a	348 ± 49 B ab	131 ± 28 B ab	3343 ± 129 C b	7919 ± 787 B ab
	0.02	574 ± 120 C c	561 ± 44 C c	2625 ± 267 B b	101 ± 10 B b	305 ± 40 B b	106 ± 12 B b	4352 ± 772 B b	8625 ± 822 B bc
	0.38	874 ± 52 A ab	905 ± 64 A a	3748 ± 883 A a	146 ± 30 A a	625 ± 133 A a	202 ± 38 A a	5539 ± 800 A a	12039 ± 1428 A a
3 days	Untreated	754 ± 178 B a	705 ± 187 A a	2667 ± 167 B a	112 ± 13 A a	309 ± 64 B ab	131 ± 28 B ab	4451 ± 879 A a	9127 ± 1020 A a
	0.02	1252 ± 320 A b	866 ± 404 A b	2755 ± 639 B b	125 ± 27 A b	341 ± 71 B ab	154 ± 44 AB ab	4075 ± 774 A b	9568 ± 1577 A b
	0.38	804 ± 17 B b	819 ± 54 A a	3575 ± 680 A a	138 ± 27 A ab	516 ± 66 A ab	194 ± 24 A a	4795 ± 926 A ab	10841 ± 1027 A ab
5 days	Untreated	550 ± 118 C b	521 ± 154 C ab	1810 ± 131 B b	103 ± 21 B a	412 ± 108 A a	104 ± 24 B b	4102 ± 617 B ab	7603 ± 1009 C b
	0.02	1497 ± 221 A a	1311 ± 101 A a	3933 ± 783 A a	168 ± 33 A a	391 ± 72 A a	169 ± 29 A a	5831 ± 261 A a	13300 ± 1094 A a
	0.38	1039 ± 225 B a	795 ± 145 B a	3212 ± 592 A a	119 ± 28 B ab	463 ± 62 A b	139 ± 38 AB b	3628 ± 622 B c	9396 ± 1046 B c

Table 2.B. *Carotenoid profile of tomato as affected by PEF processing and post-treatment storage conditions (12ºC)*

Temperature (12 ºC)									
Time of storage	Treatments (kJ/kg)	Carotenoids concentration (µg/kg of tomato)							
		Phytoene	Phytofluene	Lycopene	δ-carotene	Lutein	γ-carotene	β-carotene	Total carotenoids
Just treated	Untreated	566 ± 108 B b	505 ± 148 B b	1984 ± 471 B a	112 ± 16 A b	255 ± 117 B a	146 ± 28 B b	3686 ± 653 B ab	7254 ± 785 B b
	0.02	575 ± 94 B b	558 ± 110 B d	2305 ± 1036 B b	114 ± 21 A b	328 ± 48 B c	160 ± 41 AB b	3822 ± 753 B b	7749 ± 935 B b
	0.38	711 ± 198 A b	807 ± 259 A b	3070 ± 630 A b	112 ± 21 A b	693 ± 83 A b	180 ± 42 A c	4576 ± 675 A b	10038 ± 989 A b
1 day	Untreated	621 ± 142 B b	577 ± 115 B b	2567 ± 794 B a	118 ± 17 A b	247 ± 88 B a	131 ± 20 B b	3273 ± 756 B b	7534 ± 1089 B ab
	0.02	614 ± 99 B b	716 ± 139 AB c	1795 ± 389 B b	119 ± 13 A b	256 ± 47 B bc	167 ± 38 B ab	3794 ± 499 B b	7461 ± 932 B b
	0.38	818 ± 73 A b	852 ± 166 A b	4366 ± 583 A a	104 ± 7 A b	836 ± 86 A a	393 ± 87 A a	5800 ± 296 A a	13169 ± 747 A a
3 days	Untreated	1099 ± 159 B a	954 ± 176 B a	2637 ± 363 AB a	156 ± 31 A a	312 ± 67 A a	169 ± 33 B ab	4018 ± 418 B ab	9345 ± 1078 B a
	0.02	1431 ± 281 A a	1100 ± 113 B b	4671 ± 287 A a	167 ± 17 A a	416 ± 126 A a	203 ± 40 B ab	4975 ± 1000 A a	12962 ± 390 A a
	0.38	1679 ± 245 A a	1532 ± 242 A a	2208 ± 294 B c	177 ± 36 A a	381 ± 89 A c	265 ± 50 A b	4170 ± 663 AB b	10411 ± 624 AB b
5 days	Untreated	1163 ± 147 B a	1015 ± 102 B a	2558 ± 561 C a	173 ± 5 A a	241 ± 31 B a	182 ± 39 A a	4089 ± 413 B a	9421 ± 932 B a
	0.02	1600 ± 217 A a	1321 ± 183 A a	6389 ± 396 A a	166 ± 29 A a	330 ± 51 A ab	196 ± 25 A ab	4910 ± 416 A a	14912 ± 845 A a
	0.38	825 ± 158 C b	660 ± 137 C b	3349 ± 764 B b	91 ± 17 B b	351 ± 60 A c	184 ± 26 A c	4766 ± 598 A b	10226 ± 999 B b

Table 2.C. *Carotenoid profile of tomato as affected by PEF processing and post-treatment storage conditions (20ºC)*

Time of storage	Treatments (kJ/kg)	Temperature (20 ºC)							
		Carotenoids concentration (µg/kg of tomato)							
		Phytoene	Phytofluene	Lycopene	δ-carotene	Lutein	γ-carotene	β-carotene	Total carotenoids
Just treated	Untreated	566 ± 108 B c	505 ± 148 B c	1984 ± 471 B a	112 ± 16 A b	255 ± 117 B a	146 ± 28 B b	3686 ± 653 B a	7254 ± 785 B a
	0.02	575 ± 94 B d	558 ± 110 B c	2305 ± 1036 B b	114 ± 21 A b	328 ± 48 B b	160 ± 41 AB a	3822 ± 753 B a	7749 ± 935 B b
	0.38	711 ± 198 A b	807 ± 259 A ab	3070 ± 630 A a	112 ± 21 A b	693 ± 83 A a	180 ± 42 A a	4576 ± 675 A a	10038 ± 989 A a
1 day	Untreated	972 ± 202 A b	727 ± 181 AB b	1817 ± 371 B ab	133 ± 33 A ab	157 ± 89 B a	169 ± 37 A ab	3377 ± 679 A a	7351 ± 913 A a
	0.02	1012 ± 48 A b	818 ± 62 A b	2441 ± 409 A b	125 ± 23 A ab	283 ± 70 A a	155 ± 38 A a	3817 ± 573 A a	8650 ± 1032 A b
	0.38	575 ± 10 B bc	629 ± 74 B bc	2716 ± 605 A ab	91 ± 3 B bc	349 ± 90 A c	188 ± 40 A a	3442 ± 463 A b	7989 ± 1085 A b
3 days	Untreated	1189 ± 202 A a	1051 ± 187 A a	1429 ± 191 C b	153 ± 31 A a	191 ± 37 B a	199 ± 23 A a	3569 ± 397 A a	7782 ± 912 AB a
	0.02	790 ± 130 B c	896 ± 202 A b	2882 ± 617 A b	110 ± 11 B b	218 ± 43 B ab	169 ± 50 A a	3571 ± 267 A a	8636 ± 922 A b
	0.38	524 ± 41 C c	510 ± 121 B c	2107 ± 591 B b	85 ± 4 C c	350 ± 54 A c	167 ± 28 A a	3131 ± 274 B b	6875 ± 871 B b
5 days	Untreated	1243 ± 200 B a	1017 ± 174 B a	1479 ± 301 B b	147 ± 31 A a	260 ± 43 B a	195 ± 26 A a	3721 ± 546 B a	8062 ± 486 B a
	0.02	1498 ± 167 A a	1423 ± 303 A a	4944 ± 1081 A a	141 ± 17 A a	273 ± 64 B ab	172 ± 41 A a	3756 ± 392 AB a	12209 ± 632 A b
	0.38	973 ± 116 C a	994 ± 208 B a	2218 ± 1107 B b	139 ± 33 A a	497 ± 49 A b	215 ± 47 A a	4359 ± 551 A a	9394 ± 546 B a

Moreover, a maximum concentration in δ-carotene, lutein, γ-carotene and β-carotene was attained in tomatoes subjected to the most intense PEF treatment (0.38 kJ·kg^{-1}) and subsequently stored at 12 ºC (Table 2). The highest concentration of each individual carotenoid was reached at different storage times. Thus, highest δ-carotene concentration was reached after 3 days of storage (177 µg·kg^{-1}), while lutein, γ-carotene and β-carotene were enhanced by 238%, 200% and 77%, respectively after just 1 day. Lycopene is cyclized either to yield δ–carotene and lutein by lycopene ε-cyclase (LCYE) and lycopene β-cyclase (LYCB) or to produce γ-carotene and β-carotene by lycopene β-cyclase (LCYB) alone (Lu & Li, 2008). Therefore, the increased concentration of these minor carotenoids of tomato could be linked to the activation of genes encoding both LCYB and LYCE. Our results are in agreement with those previously reported by Vallverdú-Queralt, Oms-Oliu, et al., (2013) who proposed that PEF treatments produce the activation of LYCB and LYCE, thus resulting in an increase in β-carotene, 9-*cis*-β-carotene and lutein in tomato fruits.

4. Conclusions

The electric field strength applied and post-treatment storage conditions significantly affected the accumulation of carotenoids in tomato fruits as well as their physicochemical properties. The concentration of the major carotenoids of tomato reached their maximum values when fruits were stored at 12 ºC. Under this storage temperature, tomatoes subjected to 0.38 kJ·kg^{-1} exhibited the fastest accumulation of individual carotenoids. However, this treatment produced irreversible damage to tomato tissues, thus leading to deleterious changes in their physicochemical properties. On the other hand, mild PEF treatment conditions (0.02 kJ·kg^{-1}) led to a slower but maximum accumulation of total carotenoids (58%) and lycopene (150%) without negatively affected the quality attributes of tomato fruits. The results obtained in this study evidenced that PEF was an effective technology to stimulate the biosynthesis and accumulation of carotenoids in tomato fruits, thus enhancing their health-related properties. However, the accurate control of both the electric field

strength applied and post-treatment storage conditions is necessary for the feasible application of PEF to improve the carotenoids concentration of tomato fruits as well as of their derived products. Further studies are required in order to clarify the biochemical mechanism by which biosynthetic pathway is activated needs by PEF processing.

5. Abbreviations

PEF, pulsed electric fields; ROS, reactive oxygen species; BHT, butylated hydroxytoluene; L*, lightness; a*, green to red chromaticity; b*, blue to yellow chromaticity; hº, hue angle; TSS, total soluble solids; HPLC, high-performance liquid chromatography; ANOVA, analysis of variance; LCYB, lycopene β-cyclase; LCYE, lycopene ε-cyclase.

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CHAPTER 3

Influence of pulsed light dose spectral range on the enhancement of carotenoids content of tomato fruits

ABSTRACT

The application of pulsed light (PL) treatments is proposed as a novel technology to promote the accumulation of secondary metabolites, thus enhancing the antioxidant potential of fruits and vegetables. However, information regarding the influence of light spectrum range on the biosynthesis of carotenoids through this innovative approach is limited. This work was aimed at evaluating the effect of postharvest PL treatments of different spectral range on the carotenoids concentration as well as on the main physicochemical properties of tomato fruits during post-treatment storage. Doses of wide spectrum light (180-1100 nm), full spectrum without UV-C wavelengths (305-1100 nm) and visible (VIS) + near infrared light (NIR) (400-1100 nm) were compared. PL treatments accelerated the accumulation of both total carotenoids and lycopene concentrations in tomato fruits. Nevertheless, the efficacy of PL depended on the applied spectral range. Tomatoes subjected to PL treatments delivering wavelengths ranging from 400 to 1100 nm (VIS+NIR) exhibited the greatest enhancement in total carotenoids (31%) and lycopene (35%) concentrations after 5 days of storage. On the contrary, treatments containing UV-light did not significantly enhance carotenoid concentrations in tomatoes. On the other hand, the main physicochemical properties of tomato were not found to be affected by PL treatments, regardless the wavelength range used. These results evidenced that VIS+NIR pulsed light treatments have the potential to induce a faster accumulation of carotenoids in tomato fruits without negatively affecting their quality attributes during post-treatment storage.

1. Introduction

Tomato is one of the most important vegetable crops worldwide. Regular consumption of tomato and tomato derivatives has been associated with a lower incidence of chronic diseases, such as cancer, atherosclerosis and cardiovascular diseases (Tanumihardjo and Yang, 2010). These beneficial properties have been attributed to their high content in bioactive compounds, such as phenolic, vitamins and, especially carotenoids (Hedges and Lister, 2005). Among carotenoids, lycopene is the pigment principally responsible for the deep-red colour in ripe tomatoes, which influences the quality perception of the fruits and the derived products by consumers (Aguiló-Aguayo et al., 2013; Shi and Maguer, 2000).

Carotenoids accumulation in tomato fruits is associated to ripening and involves various physiological, morphological, biochemical and molecular changes including the transition from chloroplasts to chromoplasts (Ilahy et al., 2011). The carotenoid metabolism in tomato fruits can be affected by both genetic and environmental factors, for instance radiation intensity (Liu et al., 2015). The exposure of tomatoes to intense light doses either before or after harvest appear to have a positive effect, triggering the biosynthesis of different antioxidant compounds, including carotenoids (Poiroux-Gonord et al., 2010). This response has been linked to the induction of a photoprotective antioxidant defence response to oxidative stress, that eventually leads to the accumulation of carotenoids in tomato fruits (Aguiló-Aguayo et al., 2013).

The ability of pulsed light (PL) treatments to decontaminate fresh fruit and vegetable products without causing unacceptable modifications in their sensory and nutritional characteristics has caught the interest of researchers and processors (Soliva-Fortuny and Martín-Belloso, 2016). PL consists of pulses of intense and short-time light generated by Xenon lamps (Aguiló-Aguayo et al., 2013; Charles et al., 2013). A typical pulsed light generator system triggers a radiation of broad spectrum within the ultraviolet (shorter wavelengths) and infrared (longer

wavelengths) range (Demirci and Krishnamurthy, 2011). Each one of these radiation bands possesses different characteristics and exhibit distinct interactions with food constituents (Soliva-Fortuny and Martín-Belloso, 2016). Beyond the antimicrobial action, several research works have reported an increase in the antioxidant content of metabolically-active fruit tissues after postharvest exposure to artificial light treatments. In this regard, it has been reported that postharvest broad-spectrum PL treatments significantly increased the carotenoids concentration in tomato fruits as a consequence of the activation of their biosynthetic pathway (Aguiló-Aguayo et al., 2013; Pataro et al., 2015). Other authors have observed positive effects of low-dose UV light continuous treatments on the accumulation of carotenoids and phenolic compounds in tomatoes, resveratrol in grapes and anthocyanins in strawberries and apples (Bravo et al., 2012; Castagna et al., 2013; Liu et al., 2009; Lu et al., 2016; Soliva-Fortuny and Martín-Belloso, 2016). However, there is scarce information about the application of PL treatments with different spectral range on the accumulation of carotenoids in fruits. Therefore, this work was aimed at evaluating the effect of pulsed light dose spectral range on the accumulation of total carotenoids and lycopene as well as on the main quality attributes of tomato fruits throughout the post-treatment storage.

2. Material and methods

2.1. Reagents

Butyl hydroxytoluene (BHT) was acquired from Scharlau Chemie S.A. (Barcelona, Spain).

2.2. Tomato fruits

Tomatoes (*Lycopersicum esculentum* cv. Raf) were purchased at a wholesale distributor in Lleida (Spain) at turning stage, characterized by more than a 10% but not more than a 30% of the surface showing a definite change in colour from green to red (USDA, 1991). The fruits were stored at 12 ± 1°C until turning to a light red-stage (60-90% of

tomato surface was red) (USDA, 1991). Prior to PL treatments, tomatoes with uniform shape and size, free from fungal infection, were selected. Fruits were rinsed with tap water and carefully dried with paper cloth.

2.3. Pulsed light treatments

Pulsed light (PL) treatments were carried out using a XeMaticA-2L system (SteriBeam Systems GmbH, SteriBeam, Kehl, Germany). The sample holder consists in a polypropylene film supported by a metal framework, which was situated in a half-way between two Xenon lamps separated by a gap of 17 cm. Transparency of the film was determined by measuring the amount of energy received by a photodiode coupled to an oscilloscope and was found to be above 97% of the total emitted energy. The emitted wavelengths ranged from 180 to 1100 nm, with 15-20 % of the light in the UV region. The duration of each pulse was 0.3 ms and the fluence delivered by each lamp was 0.4 $J·cm^{-2}$ per pulse. A total energy dose of 10 $J·cm^{-2}$ per side was applied. This dose was chosen as being optimal from preliminary experiments. It is worth mentioning that this energy dose (10 $J·cm^{-2}$) is lower than the maximal cumulative treatment dose approved by FDA for treatment of food products, which is established at 12 $J·cm^{-2}$ (FDA, 2016).

To evaluate the effect of the application of light pulses of different spectrum composition, two types of filters were used: a Makrolon® polycarbonate filter which cuts off all light below 400 nm, thus allowing only the VIS and NIR to pass through, and a 2-mm thick Pyrex® glass filter that cuts all light below 305 nm allowing to pass UV-B (280-320nm), UV-A (320-400 nm), VIS and NIR wavelengths. Additionally, treatments with no filter were carried out to assess the effect of broad emitted spectrum (180-1100 nm). Namely, three treatments were compared regarding the effect on physicochemical quality parameters and total carotenoid and lycopene concentrations: wide spectrum light (180 - 1100 nm), wide spectrum light without UV-C wavelengths (305 – 1100 nm) and VIS-NIR light (400 – 1100 nm). Untreated tomatoes were used as reference. Each treatment was repeated 24 times and each

replicate comprised two tomato fruits. Immediately after PL processing, untreated and PL-treated tomatoes were stored at 12 ± 1°C in darkness. Twelve fruits were randomly withdrawn for analysis at each sampling time (0, 1, 5 and 10 days). After characterization, tomatoes from each treatment batch were ground, freeze-dried and then stored at -40ºC until carotenoid extraction.

2.4. Physicochemical properties of tomato fruits

2.4.1. Colour

The colorimetric CIELab values, $L*$ (lightness), $a*$ (red–green chromaticity) and $b*$ (blue–yellow chromaticity) were randomly measured over tomato fruits surface using a Minolta colorimeter (Minolta CR-400, Konica Minolta Sensing, Inc., Osaka, Japan). The equipment was set up for a D65 illuminant and an observation angle of 10º. A white standard plate (Y = 94.00, x = 0.3158, y = 0.3322) was used for calibration. For each assayed treatment condition and sampling time, colour parameters were determined on twelve fruits. Colour changes were expressed as $L*$ and hue angle ($hº$), which was calculated following equation 1:

$$hº = tan^{-1} \frac{b^*}{a^*} \quad (1)$$

2.4.2. Firmness

Tomato firmness was determined with a TA-XT2 texture analyser (Stable Micro Systems Ltd., Surrey, England) by measuring the maximum force required to penetrate tomato fruits with a 4-mm diameter probe to a depth of 10 cm at a rate of 5 $mm \cdot s^{-1}$. The fruits were placed so that the plunger penetrated the pericarp in the equatorial region. Results were expressed in Newtons (N).

2.4.3. pH

The pH of tomato homogenate was determined using a Crison 2001 pH-meter (Crison Instruments S.A., Alella, Barcelona, Spain).

2.4.4. Total soluble solids

Total soluble solids (TSS) content was determined by the refraction index using an Atago RX-1000 refractometer (Atago Company Ltd., Tokyo, Japan) at 25 ºC. Results were expressed as Brix degrees (ºBrix).

2.5. Carotenoids determination

2.5.1. Lycopene determination

Lycopene concentration was determined following the methodology proposed by Odriozola-Serrano et al. (2007) with slight modifications. Duplicates of 0.2 g of freeze-dried tomato samples were weighed and mixed with 20 mL of 0.05% (w/v) BHT in ethanol:hexane (4:3). The mixture was homogenized at 6 g, for 15 min and 4 ºC in a Beckman Coulter centrifuge (Avanti J-26 XP, California, United States). Then, 3 mL of distilled water were added and shaken in a vortex for 30 s. The mixture was kept at room temperature for 5 min to allow phase separation. The organic phase was collected and used to measure the lycopene concentration. The absorbance of the extract was measured at 503 nm with a microplate spectrophotometer (Thermo Scientific Multiskan GO; Vantaa, Finland). Lycopene concentration was calculated according to the following equation 2.

$$Lycopene\ concentration\,(mg \cdot kg^{-1}) = \frac{A_{503}\ x\ MW\ x\ DF\ x\ 10^{6}}{\varepsilon\ x\ L} \quad (2)$$

where A_{503} is the absorbance at 503 nm, MW is the molecular weight of lycopene (536.9 $g \cdot mol^{-1}$), DF is the dilution factor, ε is the molar extinction coefficient for lycopene (17.2×10^4 $L \cdot mol^{-1} \cdot cm^{-1}$) and L is the pathlength (cm). Lycopene concentration was expressed as $mg \cdot kg^{-1}$ of tomato.

2.5.2. Total carotenoids and chlorophylls determination

The determination of total carotenoids was carried out using the methodology proposed by Costache, Campeanu, & Neata, (2012) with slight modifications. Freeze-dried tomato samples (0.2 g) were mixed

and homogenized with 20 mL of 100% acetone in an Ultraturrax (T-25 Basic, IKA®-Werke GmbH & Co., Staufen, Germany) for 2 min in an ice-bath. Then, the mixture was centrifuged at 3000 g for 10 min and 4 ºC (Beckman Coulter, Avanti J-26 XP, California, United States) and filtered through a Whatman no. 1 paper. The extract was transferred to a 25 mL flask and the volume was adjusted with acetone. All the extractions were repeated twice. The absorbance of the extracts was measured spectrophotometrically (CECIL CE 2021; Cecil Instruments Ltd., Cambridge, UK) at the following wavelengths: 662 nm, 645 nm and 470 nm for chlorophyll *a,* chlorophyll *b* and carotenoids, respectively. The total carotenoids concentration was calculated with Eq. (3), Eq. (4) and Eq. (5).

$$C_a = 11.75\ A_{662} - 2.35\ A_{645} \quad (3)$$

$$C_b = 18.61\ A_{645} - 3.96\ A_{662} \quad (4)$$

$$C_c = (1000\ A_{470} - 2.27\ C_a - 81.4\ C_b)/227 \quad (5)$$

where C_a, C_b, and C_c stand for chlorophyll *a*, chlorophyll *b* and total carotenoid concentrations, respectively. Total chlorophylls content was calculated as the sum of chlorophyll *a* and chlorophyll *b.* Results were expressed as $mg \cdot kg^{-1}$. All procedures were performed in dim lighting and using amber glassware in order to minimize carotenoid oxidation and isomerization.

2.6. Statistical analysis

Statistical analysis was carried out using the JMP Pro v. 12.0.1 statistics software (SAS Institute, Cary, NC, USA). Differences between means were determined by analysis of variance (ANOVA). The Tukey-Kramer honestly post-hoc test was also applied. Results are expressed as mean ± standard deviation (n = 12). Moreover, a correlation analysis was performed based on the Pearson´s test. A confidence level of 95% was set up in all the analyses.

3. Results and discussion

3.1. Physicochemical characteristics

3.1.1. Colour

Colour parameters (*L** and *hº*) of untreated and PL-treated tomatoes are displayed in Figure 1. Untreated tomatoes exhibited initial *L** and *hº* values of 47.9 ± 1.4 and 71.2 ± 5.9, respectively. No significant ($p > 0.05$) differences were found in *L** and *hº* values immediately after PL treatments, regardless the applied spectral wavelength range. *L** values of untreated and PL-treated tomatoes noticeably decreased as storage progressed. However, no significant ($p > 0.05$) differences were found between untreated and PL-treated tomatoes during the storage period. Lightness is the main indicative of darkening in fruits and vegetables. In this regard, the progressive decrease in *L** values throughout storage could be associated to the accumulation of carotenoids (Arias et al., 2000). On the other hand, *hº* values significantly ($p < 0.05$) decreased during storage in both untreated and PL-treated tomatoes (Figure 1). The *hº* values of tomato fruits were not found to be significantly ($p > 0.05$) influenced by the application of PL treatments, regardless the applied spectral distribution. The *hº* values decreased through storage as a consequence of the increase of *a** values, which ranged from 8 ± 3 to 26 ± 3 at day 10 (data not shown). Similar results were reported by Pataro et al. (2015) who observed that, while tomato colour was significantly affected by the storage time, the application of PL treatments of broad spectrum did not affect the colour changes of tomato over storage. Consistently, Aguiló-Aguayo, Charles, Renard, Page, & Carlin (2013) noticed that the application of PL treatments of broad spectrum did not lead to significant differences in colour parameters of tomato fruits neither just after treatments nor during 15 days of post-treatment storage, in comparison to untreated tomatoes. In addition, Liu, Zabaras, Bennett, Aguas, & Woonton, (2009) did not found any significant influence of short burst of UV-C light, red light or sun light on lightness of tomatoes when they were treated daily for up to 21 days.

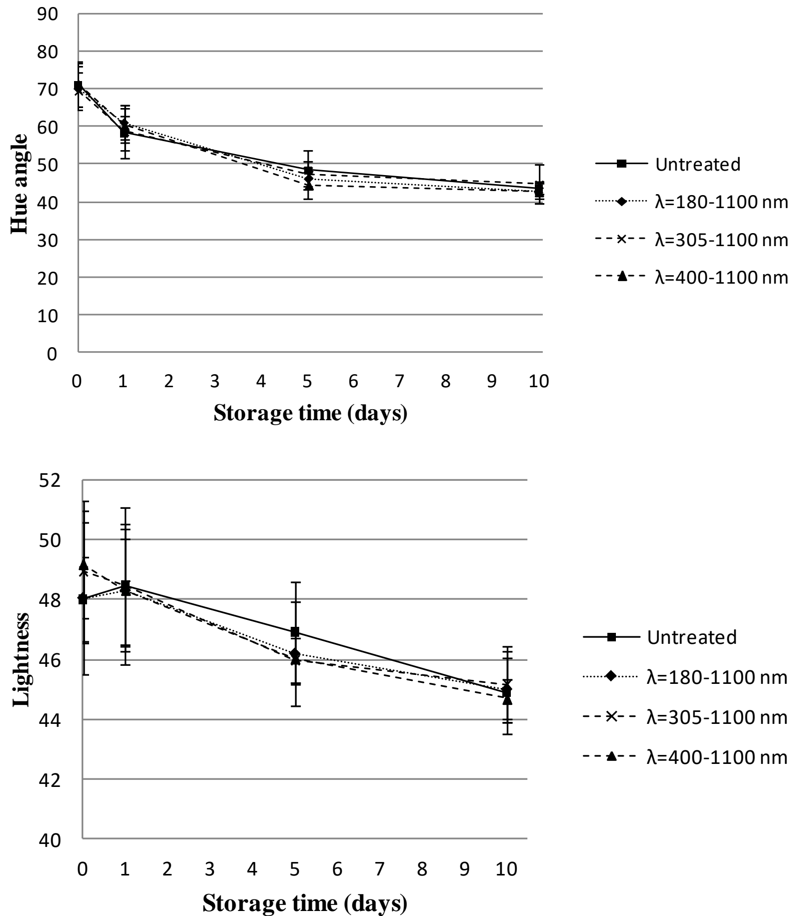

Figure 1. *Effect of PL treatments with different spectral distribution on both lightness (A) and hue angle (B) of tomato fruits stored at 12 ºC for 10 days.* Different spectral ranges were assessed: λ=180-1100 nm, λ=305-1100 nm, and λ=400-1100 nm. Data shown are mean ± standard deviation (n = 12).

3.1.2. Firmness

Changes in firmness of tomato fruits as affected by PL treatments of different spectral distribution during post-treatment storage are shown in Figure 2.

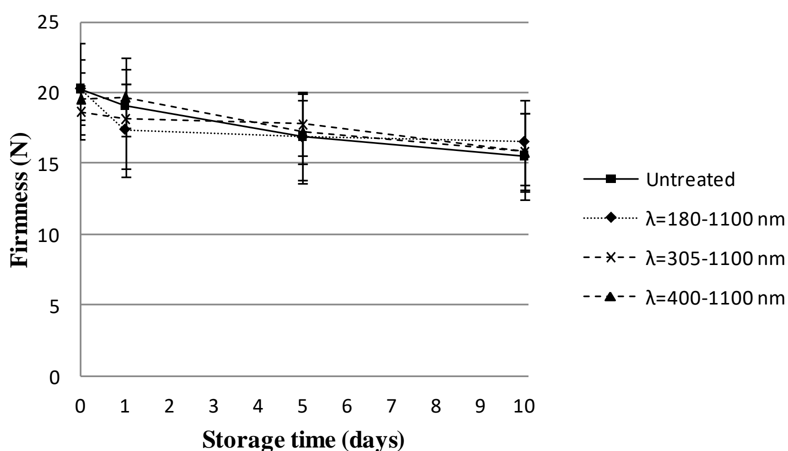

Figure 2. *Effect of PL treatments with different spectral distribution on firmness of tomato fruits stored at 12 ºC for 10 days.* Different spectral ranges were assessed: λ=180-1100 nm, λ=305-1100 nm, and λ=400-1100 nm. Data shown are mean ± standard deviation (n = 12).

A noticeable loss of firmness was observed in both untreated and PL-treated tomatoes during storage. However, firmness of tomato was not found to be significantly affected by the application of any of the assessed PL dose spectral range. Softening of tomato during storage may be mainly attributed to progressive changes in cell wall composition (Ait Barka et al., 2000). Many reports have described modifications in pectic polysaccharides during ripening, which contribute to cell wall disassembly (Osorio et al., 2011; Požrl et al., 2010). The results obtained in this study evidence that PL treatments of different spectral distribution did not affect the cell walls of tomato during post-treatment storage. In accordance with the results obtained in this work, Aguiló-Aguayo et al. (2013) did not find significant changes

in tomato firmness after the application of full-spectrum PL treatments delivering a fluence of 5.36 J·cm^{-2} compared to untreated tomatoes. In addition, other authors neither observed significant differences in firmness of tomato over 15 days of storage after the application of low-dose UV-C treatments (Liu et al. 2009). In contrast, Ait Barka et al. (2000) reported that a hormetic UV-C dose may retard fruit softening as a consequence of the decreased activity of cell-wall degrading enzymes in tomato, such as polygalacturonase, pectin methyl esterase, cellulase, xylanase, β-D-galactosidase and protease. These differences in the results seem to point out that whilst UV-C continuous light may delay tomato softening, the application of PL treatments containing different spectral wavelengths within the 180 to 1100 nm (from UV to NIR) such as those used in this work, may not have any effect on the tomato firmness during the post-treatment storage.

3.1.3. pH

The initial pH of untreated tomatoes was 4.17 ± 0.03 and was not affected just after the application of any of the PL treatments assessed (Figure 3). As storage progressed, a marked increase in pH values was observed in both untreated and PL-treated tomatoes. The variation of pH over storage began to be significant ($p < 0.05$) from the first day of storage. However, this increase was less noticeable in tomatoes subjected to PL treatments delivering wavelengths within 305-1100 nm (wide spectrum without UV-C light), thus leading to significant lower values of pH at day 10 in comparison to untreated tomatoes. The progressive increase of pH values throughout storage is usually attributed to the loss of organic acids which occurs during tomato ripening (Anthon et al., 2011). These results are consistent with those reported by other authors (Pataro et al., 2015) who have previously noticed that pH of tomato remained almost unchanged after light irradiation with UV-C and PL treatments of broad spectrum.

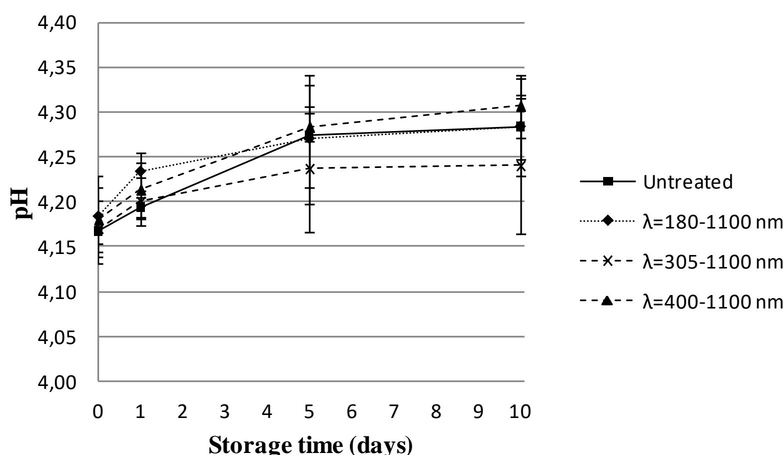

Figure 3. *Effect of PL treatments with different spectral distribution on pH of tomato fruits stored at 12 ºC for 10 days.* Different spectral ranges were assessed: λ=180-1100 nm, λ=305-1100 nm, and λ=400-1100 nm. Data shown are mean ± standard deviation (n = 12).

3.1.4. Total soluble solids

The initial TSS content of untreated tomatoes was 4.50 ± 0.06 ºBrix and was not significantly ($p > 0.05$) affected by any of the PL treatments studied (Figure 4). TSS of both untreated and PL-treated tomatoes continuously increased during storage, which may be associated to the accumulation of soluble sugars during tomato ripening (Anthon et al., 2011). However, TSS of tomato fruits were not found to be significantly ($p > 0.05$) affected by the application of PL treatments of different spectral wavelength range throughout the storage period. Although there are no previous studies regarding the influence of PL treatments of different spectral range on the TSS content of tomato, some authors have reported that the exposure of tomatoes to continuous UV-C irradiation and PL treatments of broad spectrum did not significantly influence the TSS of the fruits (Liu et al., 2009; Pataro et al., 2015), which is in line with our results.

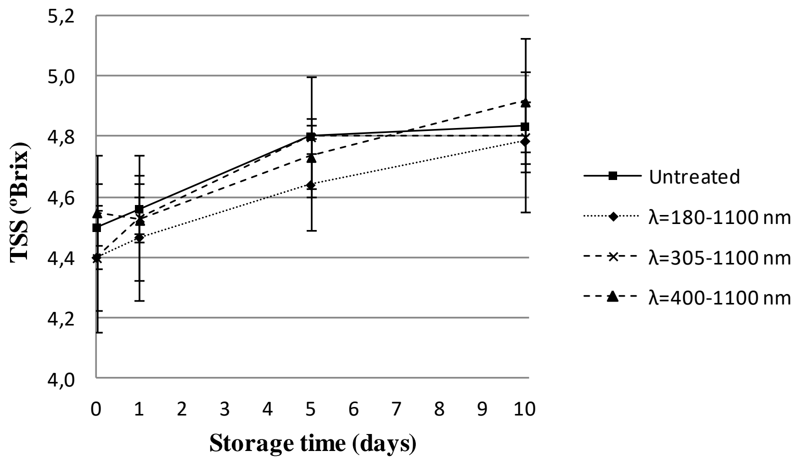

Figure 4. *Effect of PL treatments with different spectral distribution on total soluble solids (TSS) content of tomato fruits stored at 12 ºC for 10 days.* Different spectral ranges were assessed: λ=180-1100 nm, λ=305-1100 nm, and λ=400-1100 nm. Data shown are mean ± standard deviation (n = 12).

3.2. Chlorophyll content

Changes in chlorophyll content in tomato fruits as affected by the application of PL treatments of different spectral distribution are shown in Figure 5. A significant ($p < 0.05$) decreased in total chlorophyll content in both untreated and PL-treated tomatoes was observed throughout storage. Initially, total chlorophyll concentration in untreated tomatoes was 4.4 ± 0.7 $mg \cdot kg^{-1}$, which progressively decreased by 28% over storage. In contrast, PL-treated tomatoes exhibited a marked decrease in chlorophyll content during the first day of storage. Afterwards, its concentration remained almost unchanged until the end of storage. This trend was especially evident in tomatoes subjected to PL treatments with broad spectrum light (180-1100 nm), which exhibited a 30% decrease in chlorophyll concentration at day 1 in comparison to untreated tomatoes. During tomato ripening, chlorophylls disappear and in turn the accumulation of carotenoids in chromoplasts takes place (Pataro et al., 2015). This process has been

shown to be affected by several environmental factors, including light irradiance (Llorente et al., 2016). In this regard, Lopes et al., (2016) reported a delay in the loss of chlorophylls in mango pulp as affected by broad spectrum PL treatments. These results contrast with those observed in our study, suggesting that the oxidative stress response triggered by PL may differ depending on the type of fruit. Moreover, some authors have previously reported that post-harvest UV-B and UV-C irradiation significantly delayed chlorophyll degradation in tomato fruits (Maharaj et al., 1999) and broccoli (Aiamla-or et al., 2010; Ribeiro et al., 2012). These controversial results could be associated to the presence of VIS+NIR wavelengths (400-1100 nm) in the PL treatments used in this work. It was previously reported that red and far-red light are involved in chloroplast to chromoplast transition and hence, in the loss of chlorophylls (Alba et al., 2000).

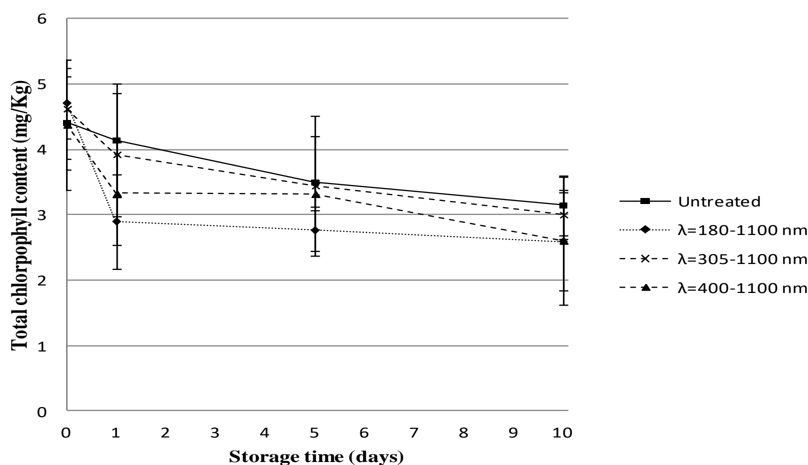

Figure 5. *Effect of PL treatments with different spectral distribution on the content of total chlorophylls of tomato fruits stored at 12 ºC for 10 days*. Different spectral ranges were assessed: λ=180-1100 nm, λ=305-1100 nm, and λ=400-1100 nm. Data shown are mean ± standard deviation (n = 12).

3.3. Carotenoids content

The effect of PL treatments of different spectral wavelength distribution on total carotenoids and lycopene contents throughout post-treatment storage is displayed in Figure 6A and B, respectively. Total carotenoids concentration of untreated tomatoes continuously increased during storage, from 19 ± 2 $mg \cdot kg^{-1}$ to 41 ± 6 $mg \cdot kg^{-1}$ at day 10. Lycopene concentration followed an upward similar trend to that observed for total carotenoids. Thus, initial lycopene concentration in untreated tomatoes was 9.7 ± 1.2 $mg \cdot kg^{-1}$ and continuously increased by 2.36-fold over the reported storage period. PL-treated tomatoes exhibited a sharp increase in total carotenoid (8-31%) and lycopene (13-35%) contents between days 1 and 5 following the treatments, regardless the applied condition, and then remained almost unchanged through further storage. Nevertheless, differences in light spectral range differently affected the final carotenoids concentration. In this regard, tomatoes subjected to PL treatments delivering wavelengths within the 400 to 1100 nm (VIS + NIR) exhibited a maximum 31% increase in total carotenoids concentration after 5 days of storage. After such PL treatments, lycopene content also attained its maximum enhancement (1.35-fold increase) at day 5. However, treatments applying light containing UV fractions did not exert any significant ($p > 0.05$) influence on the accumulation of total carotenoids and lycopene in tomato fruits during storage, in comparison to untreated tomatoes. It was reported that fruit-localized phytochromes play a fundamental role in the light-induced carotenoids biosynthesis in tomatoes (Alba et al., 2000). Phytochromes are photoreceptors involved in response-regulation by red light (wavelength 660 nm) and far-red light (wavelength 730 nm) (Llorente et al., 2016; Schofield and Paliyath, 2005). In this regard, the fast accumulation of carotenoids in tomatoes after the application of PL treatments was likely to be due to the activation of carotenogenesis triggered by red and far-red light. This is in accordance to Gautier et al. (2008) who reported that red light is involved in the modulation of the phytoene synthase (PSY) activity, which is considered the first committed step of carotenoids biosynthesis.

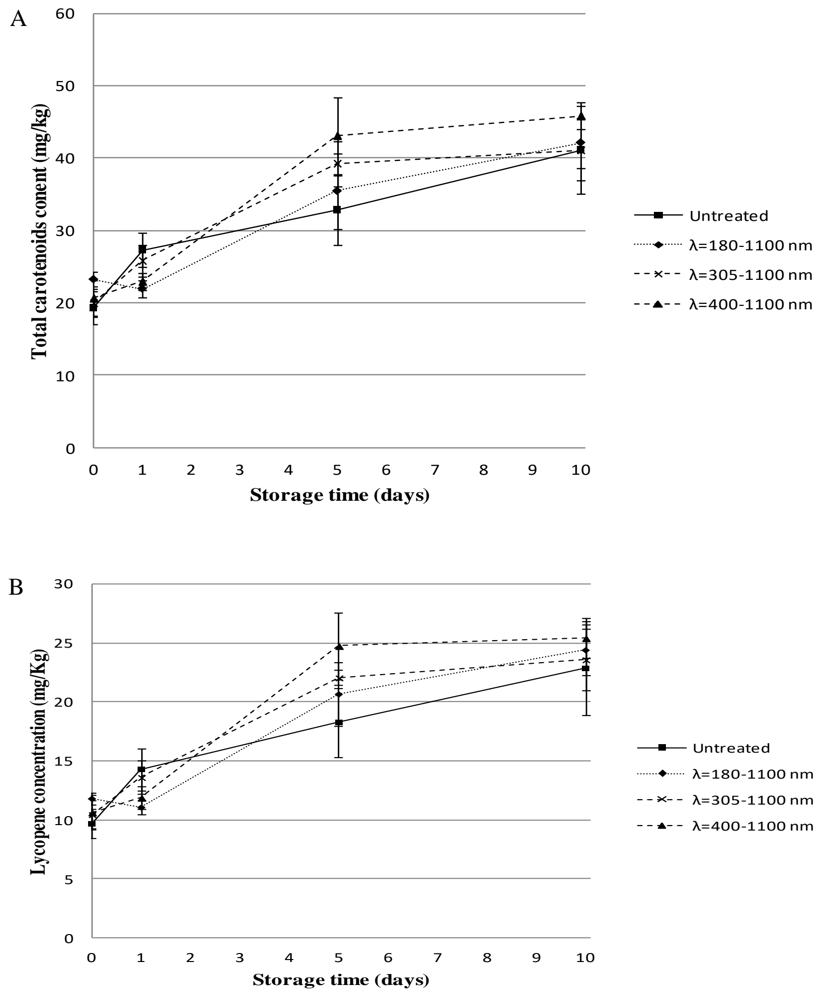

Figure 5. *Effect of PL treatments with different spectral distribution on the content of total carotenoids (A) and lycopene (B) of tomato fruits stored at 12 ºC for 10 days.* Different spectral ranges were assessed: λ=180-1100 nm, λ=305-1100 nm, and λ=400-1100 nm. Data shown are mean ± standard deviation (n = 12).

The differences observed on the carotenoids concentration when PL treatments of different spectral range were applied could be associated to the deleterious effect of UV-light (180-400 nm). It has been reported that UV-light exposure accounts for the formation of free radicals which lead to the initiation of photooxidation followed by photodescomposition (Demirci and Krishnamurthy, 2011). These processes could be behind the modification of both the enzymes involved in the carotenoid biosynthesis and some food constituents, leading to product quality deterioration (Bravo et al., 2012; Demirci and Krishnamurthy, 2011; Pataro et al., 2015). In this regard, Tiecher, de Paula, Chaves, & Rombaldi, (2013) and Lu et al., (2016) noticed that the application of UV-C light delayed the carotenoids accumulation in tomato fruits. It is know that tomato ripening is triggered by an increase in ethylene production, which is related with a rapid accumulation of lycopene (Liu et al., 2015). However, Lu et al. (2016) noticed that UV-C light postponed the ethylene production, resulting in delayed lycopene bioproduction in tomato fruits. In addition, C. Liu et al., (2011) reported that the application of postharvest UV-B radiation with a fluence ranging from 10 to 40 $kJ \cdot m^{-2}$ significantly reduced the lycopene content of tomato fruits. Therefore, these effects can probably counteract the beneficial effect of both red and far-red light on the activation of the carotenoids biosynthesis and their accumulation.

Moreover, the inverse and significant correlation found between chlorophylls and both total carotenoids and lycopene concentration ($R = 0.709$ and $R = 0.71$, $p < 0.001$, respectively) indicates that PL treatments may accelerate the degradation of chlorophylls and the synthesis and accumulation of carotenoids, mainly lycopene, in tomato fruits. In accordance with the results obtained by other authors (Bravo et al., 2012; Liu et al., 2009; Pataro et al., 2015), this study suggest that the exposure to PL can significantly stimulate the accumulation of these health-related compounds during post-treatment storage. However, the accurate control of the spectral wavelength range is necessary in order to optimize the induced-accumulation of carotenoids in tomato fruits by the application of PL treatments.

4. Conclusion

PL treatments have the potential to induce a faster accumulation of carotenoids in tomato fruits without negatively affecting their quality attributes (colour, firmness, pH and total soluble solids) during post-treatments storage. The stimulation of the carotenoid biosynthetic pathway of the fruits can be optimized under selected spectral wavelength range. A proper combination of dose spectral range and storage time is necessary to achieve positive effects. The efficiency of the emitted spectrum wavelengths increased as follows: UV + VIS + NIR < wide spectrum light without the UV-C range < VIS + NIR. Especially effective were those treatments containing only VIS and NIR fractions (400-1100nm), which led to a 1.31- and 1.35-fold increase in total carotenoids and lycopene concentrations, respectively, in comparison to untreated tomatoes. Further studies focussing on the effects of PL treatments with different spectral range on the tomato metabolism should be carried out in order to gain knowledge regarding the processes associated to the changes in the antioxidant potential of tomato fruits.

5. Abbreviations

PL, pulsed light; ultraviolet light, UV; visible light, VIS; near-infrared light, NIR; TSS, total soluble solids; C_a, chlorophyll a; C_b, chlorophyll b; C_c total carotenoids concentration; L* (lightness), a* (red–green chromaticity); b* (blue–yellow chromaticity); hº, hue angle

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CHAPTER 4

In vitro bioaccessibility of carotenoids in tomato derivatives as affected by ripeness stage and the addition of different types of oil

ABSTRACT

The bioaccessibility of tomato carotenoids is known to be influenced by several factors, including the fruit ripeness stage and the characteristics and composition of the derived products. However, little information is available regarding the reciprocal influence of these parameters on the carotenoids bioaccessibility. The simultaneous effect of tomato ripeness stage (mature green, pink and red-ripe), mechanical processing (cutting and grinding) and oil addition (coconut, sunflower, and olive oils) on the amount and bioaccessible fraction of carotenoids were evaluated. Greatest concentration of carotenoids were found in tomato products obtained from fruits processed at the most advanced ripeness stage, which also led to an increase in their bioaccessibility. 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, their bioaccessibility was greater 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.

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.

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 & 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 (Bohn 2008). 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 (Svelander and others 2010).

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 (Bohn 2008). 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; Huo and others 2007; 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; Huo and others 2007).

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) and 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 10º 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}.

The results of the physicochemical characteristics of tomatoes at the three selected ripeness stages are shown in Table 1.

Table 1. *Physicochemical characterization of tomato at different ripeness stages.*

Parameter	Ripeness stage		
	Mature-green	Pink	Red
*L**	45.9 ± 2.8 ab	46.9 ± 1.8 a	44.9 ± 2.8 b
*a**	-13.8 ± 1.5 c	1.7 ± 3.8 b	15.0 ± 2.9 a
*b**	25.2 ± 1.9 a	24.9 ± 2.4 a	23.8 ± 2.8 a
a/b**	-0.6 ± 0.1 c	0.1 ± 0.2 b	0.6 ± 0.2 a
Soluble solids (ºBrix)	4.77 ± 0.15 a	4.85 ± 0.14 a	5.05 ± 0.07 a
pH	4.09 ± 0.13 a	4.02 ± 0.02 a	4.07 ± 0.03 a
Titratable acidity (g citric acid · kg^{-1})	0.45 ± 0.06 a	0.46 ± 0.05 a	0.45 ± 0 a

Values are expressed as mean ± standard deviation (n = 4).

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. Tomato cubes and tomato puree were then prepared. 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-cm^3 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. The specific methodology used is explained hereafter.

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 $CaCl_2 \cdot 2H_2O$, 0.4 g·L^{-1} of KCl, 0.06 g·L^{-1} of KH_2PO_4, 0.1 g·L^{-1} of $MgCl_2 \cdot 6H_2O$, 0.049 g·L^{-1} of $MgSO_4 \cdot 7H_2O$, 8 g·L^{-1} of NaCl, 0.35 g·L^{-1} of $NaHCO_3$ and 0.048 g·L^{-1} of

Na_2HPO_4 (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 $g{\cdot}L^{-1}$ in 0.1 M HCl) was added to assure a final concentration of 1.8 $g{\cdot}L^{-1}$ in the gastric digesta. Finally, pH was adjusted to 2 with 5 M HCl. The mixture was incubated for 120 min at 37 °C 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 $g{\cdot}L^{-1}$ of pancreatin from porcine pancreas and 25 $g{\cdot}L^{-1}$ of bile extract from porcine were dissolved in 0.1 M $NaHCO_3$. It was added into the small intestinal digesta to provide final concentrations of 0.4 $g{\cdot}L^{-1}$ and 2.5 $g{\cdot}L^{-1}$, 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 °C (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 ºC until analysis.

2.6. Determination of carotenoids

2.6.1. Extraction

The lipophilic fraction was extracted according to the procedure described by Rodríguez-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 $g \cdot L^{-1}$) 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):

$$\textit{Total carotenoids } (mg \cdot kg^{-1}) = \frac{A_{470} \ x \ V \ x \ 10^4}{A_{1cm}^{1\%} \ x \ G} \qquad (1)$$

where A_{470} is the absorbance at 470 nm, V is the total volume of extract (mL), $A_{1cm}^{1\%}$ is the absorption coefficient (absorbance at a given wavelength of a 1% solution in spectrophotometer cuvette with a 1-cm light path) of a mixture of carotenoids established in 2500 according to Gross (1991), and G is sample weight (g). Total carotenoids results were expressed in $mg \cdot kg^{-1}$ 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.

$$Lycopene\ (mg \cdot kg^{-1}) = \frac{A_{503}\ x\ MW\ x\ DF\ x\ 10^{6}}{\varepsilon\ x\ L} \quad (2)$$

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

$$Bioaccessibility\ (\%) = \frac{BC_{digested}}{BC_{undigested}} x100 \quad (3)$$

where $BC_{digested}$ corresponded to the overall concentration of bioactive compound in the micellar fraction and $BC_{undigested}$ was the concentration in the non-digested samples.

2.8. Statistical analysis

Tomato processing and the *in vitro* gastrointestinal digestion were conducted in duplicate, whereas each analysis was conducted twice (n = 4). Results were reported as the mean ± standard deviation. Analysis of variance (ANOVA) of the results was carried out using JMP

Pro v.12.0.1 software. The honestly significant difference (HSD) test of Tukey was used to determine significant differences ($p < 0.05$) between each studied parameter in this research (tomato ripening, type of processing and addition of oil). Correlation analysis based on Pearson´s test was carried out in order to determine the relationship between each assayed parameter.

3. Results and discussion

3.1. Carotenoids content

Carotenoid concentration in tomato derivatives was strongly influenced by the ripeness stage of the raw fruits. Total carotenoid content (TCC) in tomato-based products markedly increased as fruits ripened, ranging from 0.53 ± 0.11 $mg \cdot kg^{-1}$ at mature-green stage to 14.82 ± 1.62 $mg \cdot kg^{-1}$ 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 $mg \cdot kg^{-1}$ 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 colourful 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.

Table 2. *Changes of total carotenoids and lycopene contents (mg · kg^{-1}) of two tomato derivatives (cubes and puree) when different types of oil were added (coconut, olive and sunflower oils) during ripening.*

Ripeness stage	Oil type	Tomato cubes		Tomato puree	
		Total carotenoids	Lycopene	Total carotenoids	Lycopene
Mature-green	No oil	1.27 ± 0.24 $^{e\ B}$	0.21 ± 0.04 $^{d\ BC}$	0.53 ± 0.11 $^{d\ D}$	0.11 ± 0.02 $^{d\ C}$
	Coconut oil	2.02 ± 0.12 $^{e\ A}$	0.43 ± 0.01 $^{d\ A}$	0.88 ± 0.07 $^{d\ CD}$	0.25 ± 0.05 $^{d\ B}$
	Olive oil	2.02 ± 0.25 $^{e\ A}$	0.37 ± 0.08 $^{d\ A}$	1.35 ± 0.08 $^{d\ B}$	0.31 ± 0.05 $^{d\ AB}$
	Sunflower oil	1.92 ± 0.14 $^{e\ A}$	0.33 ± 0.02 $^{d\ AB}$	1.13 ± 0.07 $^{d\ BC}$	0.27 ± 0.04 $^{d\ B}$
Pink	No oil	7.52 ± 0.40 $^{c\ A}$	3.49 ± 0.43 $^{c\ A}$	4.76 ± 0.28 $^{c\ C}$	2.12 ± 0.22 $^{c\ CD}$
	Coconut oil	6.67 ± 0.96 $^{cd\ AB}$	3.19 ± 0.54 $^{c\ AB}$	4.09 ± 0.36 $^{c\ C}$	1.78 ± 0.16 $^{c\ D}$
	Olive oil	5.31 ± 0.17 $^{d\ BC}$	2.63 ± 0.12 $^{c\ BC}$	5.14 ± 0.58 $^{c\ BC}$	2.43 ± 0.21 $^{c\ BCD}$
	Sunflower oil	6.45 ± 1.28 $^{cd\ AB}$	2.95 ± 0.62 $^{c\ ABC}$	5.07 ± 0.79 $^{c\ BC}$	2.42 ± 0.31 $^{c\ BCD}$
Red	No oil	14.82 ± 1.62 $^{a\ A}$	8.07 ± 0.87 $^{a\ A}$	7.94 ± 0.88 $^{b\ D}$	4.48 ± 0.78 $^{b\ D}$
	Coconut oil	11.44 ± 0.24 $^{b\ B}$	6.33 ± 0.12 $^{b\ BC}$	10.19 ± 0.35 $^{a\ BC}$	5.31 ± 0.19 $^{a\ BCD}$
	Olive oil	11.53 ± 0.55 $^{b\ B}$	6.39 ± 0.29 $^{b\ BC}$	8.73 ± 0.99 $^{b\ CD}$	4.67 ± 0.64 $^{ab\ D}$
	Sunflower oil	11.39 ± 0.94 $^{b\ B}$	6.46 ± 1.28 $^{b\ B}$	8.55 ± 0.44 $^{b\ CD}$	4.91 ± 0.42 $^{ab\ CD}$

Values are expressed as mean ± standard deviation (n = 4). 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 ($p < 0.05$).

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 was in accordance with those found by Ilahy and others (2011) who also reported a continuous increase in TCC and LC during tomato ripening. It has been described a number of physiological, morphological and biochemical changes during tomato ripening, including chlorophylls degradation and biosynthesis and accumulation of carotenoids, especially lycopene, during chloroplast to chromoplast transition (Ilahy and others 2011; Hdider and othres 2013). The activation of the genes encoding the enzymes, such phytoene synthase, and the decreased activity of lycopene β- and ε-cyclase, are thought to be the responsible of the massive accumulation of lycopene in tomato fruits during ripening, and hence in their derivative products (Ilahy and others 2011; Liu and others 2015).

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. This is consistent with results obtained by Takeoka and others (2001) who reported significant losses in the concentration of lycopene when tomatoes were processed into paste. 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 otrhers 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 any of the studied 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 suggest that oils may play a protective role against carotenoids degradation. 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 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.

3.2. 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. Generally, LB showed lower values than TCB. This is in line with the results reported by Huo and others (2007) who confirmed that the efficiency of micellarization of carotenoids and, therefore, their chance to be bioaccessible was inversely proportional to their hydrophobicity. As lycopene is the most hydrophobic carotenoid, due to its molecular configuration, its dispersion in the aqueous micellar phase is limited, thus leading to the least bioaccessibility values.

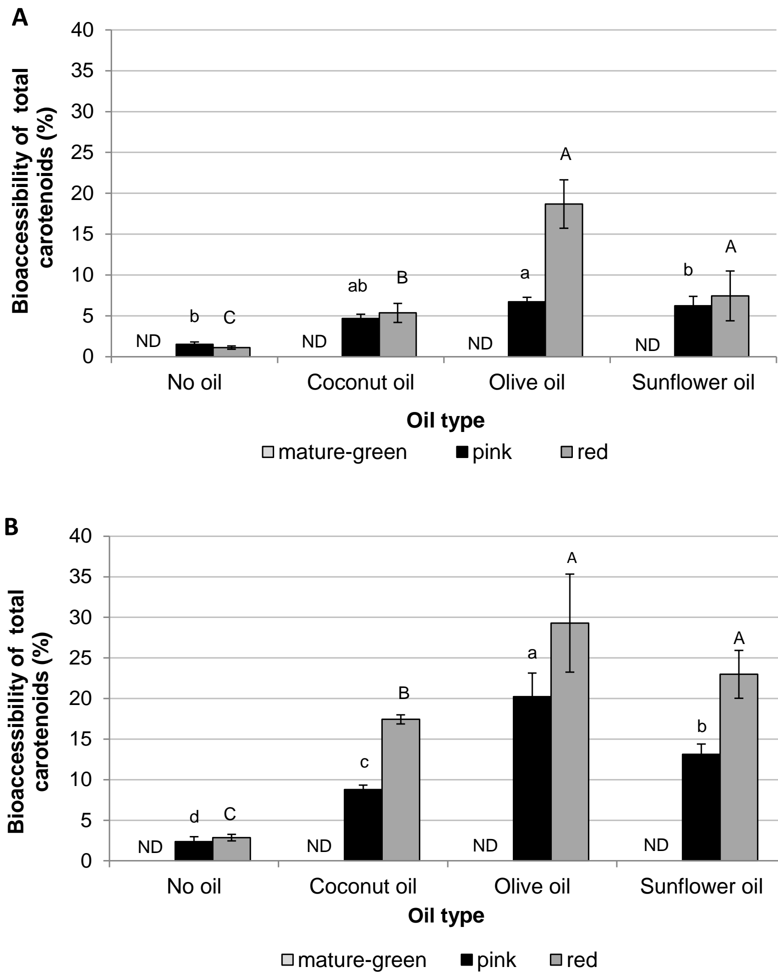

Figure 1. Total carotenoid bioaccessibility (%) in tomato cubes (A) and tomato puree (B) processed at three ripeness stages (mature-green, pink and red-ripe) after the addition of 5% of different types of oil (coconut, olive and sunflower oil). Results were expressed as mean ± standard deviation. Different 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.

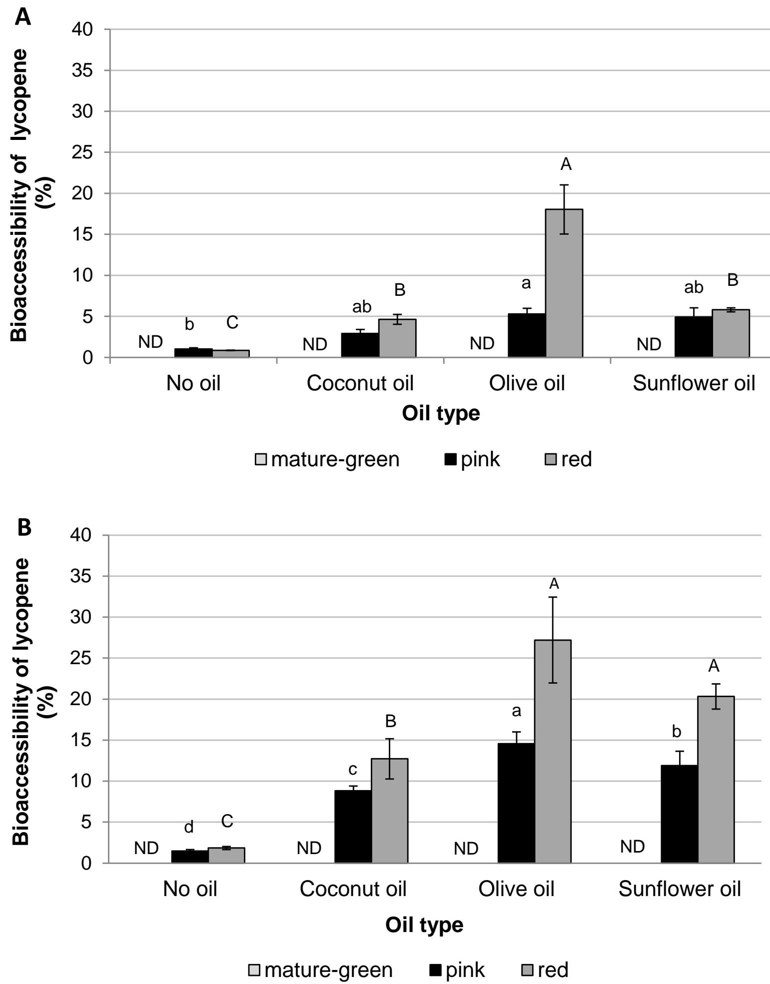

Figure 2. Lycopene bioaccessibility (%) in tomato cubes (A) and tomato puree (B) processed at three ripeness stages (mature-green, pink and red-ripe) after the addition of 5% of different types of oil (coconut, olive and sunflower oil). Data represents average values ± standard deviation. Different lower 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.

In addition, other authors have reported that lycopene is less bioaccessible than other carotenoids such as β-carotene or α-carotene due to differences in the chemical structure of these compounds (Ryan and others 2008; Svelander and others 2011).

The bioaccessibility of carotenoids was influenced by the stage of ripeness, the degree of tissue disruption of tomato derivatives and the type of added oil (Figure 1 and Figure 2). 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 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 another 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). 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, leading to fruit softening (Paniagua and others 2014). Therefore, tissue softening might increase the bioaccessibility of carotenoids due to the reduction of pectin present in the final digesta as a consequence of its depolymerization. In addition, this process could also facilitate the disruption of cell membranes 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, breaking down cell membranes and disassociating the carotenoid-protein complexes (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. 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). According to Colle and others (2010), when intense mechanical treatments are applied, carotenoids were less protected by the tomato matrix and might consequently be more sensitive to isomerization. In

this regard, tomato grinding could favor the conversion of all-*trans* isomers to *cis*-forms, which is thought to be preferentially absorbed by enterocytes (Boileau and others 2002). In this regard, it has been reported that c*is*-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 (Huo and others 2007). 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 its 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 and others 2012; Huo and others 2007; Nagao and others 2013). 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.

4. 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.

5. Abbreviations

DPPH, 2,2-diphenil-1-picrylhydrazyl; 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.

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CHAPTER 5

Application of pulsed electric fields to tomato fruits for enhancing the concentration and bioaccessible fraction of carotenoids in a derived product

ABSTRACT

The application of pulsed electric fields (PEF) to whole tomatoes is proposed as a pre-processing treatment to obtain purees with high health-related properties. Tomato fruits were subjected to different electric field strengths (0.4, 1.2 and 2 $kV \cdot cm^{-1}$) and number of pulses (5, 18 and 30 pulses). Tomatoes were stored at 4ºC for 24 h after PEF processing and then ground and mixed with 5% of olive oil. The resulting oil-added purees were subjected to an *in vitro* gastrointestinal digestion. PEF treatments significantly increased the amount and bioaccessible fraction of carotenoids in the oil-added tomato purees. Treatments conducted at 2 $kV \cdot cm^{-1}$ and 30 pulses led to the greatest enhancement in the concentration of any of the carotenoids studied in the oil-added tomato puree. Bioaccessibility of lycopene, δ-carotene, β-carotene, γ-carotene and lutein was increased by 132%, 2%, 53%, 527% and 125%, respectively, in oil-added purees obtained from tomatoes subjected to 5 pulses at 2 $kV \cdot cm^{-1}$.

Results evidenced that the application of PEF treatments to tomato fruits can enhance the amount of total and individual carotenoids as well as their bioaccessible fraction in the derived product. Therefore, the application of PEF as a pre-treatment could be considered as a promising technology to obtain tomato derivatives with high antioxidant potential.

1. Introduction

Several epidemiological studies have concluded that the increased consumption of tomato and tomato derivatives is associated with lower rates of age-related macular degeneration and cataract, better immune response, as well as lower risk of cardiovascular diseases and certain types of cancer (Tanumihardjo & Yang, 2010). These beneficial properties of tomato are often related to the presence of high amount of carotenoids, which are lipophilic phytonutrients that are efficient singlet oxygen quenchers, and hence effective antioxidants (Colle, Lemmens, Van Buggenhout, Van Loey, & Hendrickx, 2010; Svelander et al., 2010).

Over the last decades, pulsed electric fields (PEF) have emerged as a non-thermal technology with several potential applications in food processing. During PEF treatments, food tissues are subjected to an external electrical field for a few microseconds, which induces local structural changes and eventually causes the breakdown of cell membranes (Toepfl, Heinz, & Knorr, 2005). Based on this process, called electropermeabilization, PEF can be exploited for different goals, e.g. the inactivation of microorganisms (Álvarez, Condón, & Raso, 2006) and quality-related enzymes (Martín-Belloso & Elez-Martínez, 2005) and the improvement of both osmotic dehydration processes (Barba et al., 2015) and extraction of intracellular metabolites (Zderic, Zondervan, & Meuldijk, 2013). In addition, the use of PEF treatments has been recently proposed to induce stress reactions in metabolically active plants at the cellular level (Soliva-Fortuny, Balasa, Knorr, & Martín-Belloso, 2009; Vallverdú-Queralt, Oms-Oliu, et al., 2013). These stress reactions are thought to activate a wide range of metabolic pathways that lead to the accumulation of secondary metabolites involved in the defense response of plants against both biotic and abiotic stress conditions (Balasa & Knorr, 2011; Vallverdú-Queralt et al., 2012; Vallverdú-Queralt, Oms-Oliu, et al., 2013). In a previous study, Vallverdú-Queralt et al. (2013) proposed the application of PEF treatments to enhance the amount of carotenoids in tomato fruits as well as in tomato juices obtained from PEF-treated fruits. However, the

effect of PEF processing on the bioaccessibility of carotenoids in fruits and vegetables has been scarcely studied.

Bioaccessibility may be defined as the fraction of an ingested compound that is released from the food matrix during digestion thus becoming accessible for intestinal uptake (Fernández-García, Carvajal-Lérida, & Pérez-Gálvez, 2009). The bioaccessible fraction of bioactive compounds is more relevant than the total amount present in the original food (Knockaert et al., 2012). In this regard, the determination of bioaccessibility is accepted as an effective procedure to study the nutritional and functional potential of food products (Failla, Huo, & Thakkar, 2008). As already reported by many authors, carotenoids bioaccessibility is influenced by several factors. The matrix in which the compound is embedded, the content of dietary fat and fibre, the type and amount of carotenoid compounds as well as particle size and distribution, are among the most relevant (Amorim-Carrilho, Cepeda, Fente, & Regal, 2014). Carotenoids are naturally located in chromoplasts, which have been suggested to act as important physical structural barriers hindering the micellarization of these lipophilic compounds (Palmero, Lemmens, Hendrickx, & Van Loey, 2014). Several studies have reported that processing operations that disrupt the food matrix, may facilitate their release, transformation and absorption during digestion, thus increasing their bioaccessibility (Parada & Aguilera 2007; Svelander et al. 2010; Colle et al. 2013). Since PEF treatments produce an electric breakdown of the cell membranes, it is thought that this technology could favour the release of carotenoids from the food matrix. In this regard, Rodríguez-Roque et al. (2015) reported that the application of high intensity PEF treatments enhanced the bioaccessibility of some carotenoids in fruit-based beverages. To the best of our knowledge, no information is available regarding the bioaccessibility of carotenoids in a processed plant-based food product as affected by the application of PEF treatments to intact raw fruits. Therefore, the main objective of this work was to evaluate changes in the concentration and bioaccessible fraction of individual carotenoids in oil-added purees obtained from tomato fruits treated with different PEF conditions.

2. Material and methods

2.1. Reagents

All digestive enzymes (α-amylase from porcine pancreas, pepsin from hog stomach, pancreatin from porcine pancreas, bile extract porcine), magnesium hydroxide carbonate, 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). Lycopene, γ-carotene, δ-carotene, β-carotene, lutein, phytofluene and phytoene were obtained from Carote-Nature (Ostermundigen, Switzerland).

2.2. Tomato fruits

Tomatoes (*Lycopersicum esculentum* cv. Raf) were purchased from a local market (Lleida, Spain) at turning stage (10 – 30% of tomato surface showing red colour). The fruits were stored at 12 ºC until they reached the red-ripe stage, meaning that more than 90% of the surface had turned red (USDA, 1991). Before PEF treatments, tomatoes were rinsed with tap water and dried carefully with paper cloth.

2.3. Pulsed electric field treatments

PEF treatments were carried out using a bench scale system (Physics International, San Leandro, CA, USA) which delivers monopolar exponential-wave pulses from a capacitor of 0.1 µF at a frequency of 0.1 Hz. The treatment chamber consists of a parallelepiped container with two stainless steel parallel electrodes (200 mm x 80 mm) separated by a gap of 10 cm. A batch of tomatoes (2 fruits) was placed into the treatment chamber filled with tap water. Tomatoes were subjected to different treatments defined by electric field strength (0.4,

1.2 and 2 kV·cm^{-1}) and number of pulses (5, 18 and 30 pulses). The specific energy input corresponding to each treatment was calculated according to Soliva-Fortuny, Vendrell-Pacheco, Martín-Belloso, & Elez-Martínez, (2017) and is displayed in Table 1. Each PEF treatment was repeated twice. PEF-treated tomatoes were immediately stored at 4 ºC for 24 h, as previously described by Vallverdú-Queralt et al., (2013).

Table 1. *PEF treatment conditions and calculated specific energy inputs.*

Electric field strength (kV·cm^{-1})	Number of pulses	Specific energy input (kJ·kg^{-1})
0	0	Untreated
0.4	5	0.02
0.4	18	0.06
0.4	30	0.09
1.2	5	0.14
1.2	18	0.50
1.2	30	0.83
2	5	0.38
2	18	1.38
2	30	2.31

2.4. Preparation of tomato puree

Twenty four hours after PEF processing, tomatoes from each PEF treatment batch were cut into pieces and ground for 90 seconds in a blender (Solac Professional Mixter BV5722, Spain). Then, 5% of olive oil (w/w) was added and mixed in a grinder (Moulinex DP700G-BP, France) for 10 seconds in order to obtain a homogeneous puree. Untreated tomatoes were used as reference. An aliquot of this homogenate was directly freeze-dried and stored at −40 °C until carotenoids extraction in order to determine carotenoid profile in the non-digested samples. A second fraction was subjected to an *in vitro* gastrointestinal digestion.

2.5. *In vitro* gastrointestinal digestion

Each oil-added puree obtained from either untreated or PEF-treated tomatoes was subjected to a static *in vitro* gastrointestinal digestion consisting of oral, gastric and small intestinal phases, following the methodology previously proposed by Tagliazucchi, et al. (2012) and Rodríguez-Roque, et al. (2013) with slight modifications.

Oral phase: 75 grams of oil-added tomato puree were weighed and mixed with 75 mL of simulated salivary fluid (SSF), which contained 150-200 uds·mL^{-1} of α-amylase. The composition of SSF was 0.1854 g·L^{-1} of $CaCl_2 \cdot 2H_2O$, 0.4 g·L^{-1} of KCl, 0.06 g·L^{-1} of KH_2PO_4, 0.1 g·L^{-1} of $MgCl_2 \cdot 6H_2O$, 0.049 g·L^{-1} of $MgSO_4 \cdot 7H_2O$, 8 g·L^{-1} of NaCl, 0.35 g·L^{-1} of $NaHCO_3$ and 0.048 g·L^{-1} of Na_2HPO_4 (pH = 6.8). The mixture was homogenized in a stomacher laboratory blender (IUL Instruments, Barcelona, Spain) for 1 min in order to simulate mastication. Subsequently, it was incubated in an orbital shaker (Ovan, Badalona, Spain) at 37 ºC for 10 min and 95 rpm (Tagliazucchi et al. 2012).

Gastric phase: pH of the digesta was adjusted to 4 with 1 M HCl. Then, 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. The pH was immediately adjusted to 2 by adding 5 M HCl. The mixture was incubated at 37 °C and 95 rpm for 120 min in an orbital shaker.

Small intestinal phase: to simulate duodenal conditions, the pH of the digesta was set to 5.3 with 2 M NaOH. Then, 15 mL of pancreatin/bile solution (4 g·L^{-1} and 25 g·L^{-1} in 0.1 M $NaHCO_3$ (w/v), respectively) were added into the small intestinal digesta. The pH was then immediately adjusted to 7.5 with 2 M NaOH. The mixture was incubated at 37 ºC and 95 rpm for 120 min in an orbital shaker.

To quantify the amount of carotenoids released from tomato matrix and incorporated into the micellar fraction, the small intestinal digesta was centrifuged at 33.768 g for 20 min at 4°C (Beckman Coulter, Avanti J-26 XP, California, United States). The aqueous-micellar phase was

collected and filtered across a Whatman 1 filter paper and subsequently across a cellulose filter (1-3 µm pore size, 70 mm diameter, Filtros Anoia S.A., Barcelona, Spain) in order to eliminate any crystalline carotenoid or undigested lipid. The micellar phase was eventually freeze-dried and stored at -40 ºC until carotenoid extraction.

2.6. Quantification of carotenoids

2.6.1. Extraction

Carotenoids were extracted following the methodology proposed by Rodríguez-Roque et al. (2013) with slight modifications. Non-digested (1 g) and digested (1.5 g) freeze-dried samples were weighed and mixed with 0.1 % (w/w) magnesium hydroxide carbonate and 10 mL of 0.05% (w/v) BHT in ethanol:hexane (4:3 v/v). The mixture was homogenized using an Ultraturrax (T-25 Basic, IKA®-Werke GmbH & Co., Staufen, Germany) for 2 min in an ice-bath. Then, it was filtered once through a Whatman no.1 paper under reduced pressure. The residue was re-extracted with a second volume of 10 mL of ethanol:hexane (4:3 v/v). The mixture was again filtered and the pellet was washed twice with 5 mL of ethanol and once with 5 mL of hexane. All the filtrates were placed in an amber round-bottom flask and rotoevaporated (rotovapor R-3000, BUCH, Switzerland) at 45 ºC for 15 min to dryness. The residue was then saponified by adding 10 mL of methanolic KOH 0.5 M + 0.1% of BHT (v/w) and 10 mL of diethyl ether, under N_2 atmosphere for 30 min with continuous agitation. Afterwards, the saponified extract was placed in an amber decanting funnel and washed twice with 25 mL of 10% NaCl solution and thrice with 25 mL of distilled water. The aqueous phase was discarded after each wash. The organic phase was collected and rotoevaporated at 45 °C for 20 min to dryness. The residue was dissolved with 4 mL of diethyl ether and placed in an amber glass vial. Finally, the solvent was evaporated under N_2 atmosphere and stored at −40 °C until analysis. All the extractions were repeated twice. Prior to HPLC injection, extracts from non-digested and digested samples were reconstituted with 1 mL and 200 µL of methylene chloride, respectively, and passed through a 0.45 µm filter.

2.6.2. Analysis

Carotenoids were separated and quantified by high-performance liquid chromatography (HPLC) following the methodology reported by Odriozola-Serrano et al. (2009). The HPLC system was composed by a 600 controller and an array detector 2996 (Waters Corp.) which was set to scan from 240 to 550 nm. Carotenoids separation was performed on a reverse-phase C18 Spherisorb® ODS2 (5 µm) stainless steel column (4.6 mm x 250 mm) at room temperature with a flow rate of 0.7 $mL·min^{-1}$. An isocratic elution of acetonitrile (85%), methanol (10%), methylene chloride (3%) and hexane (2%) was maintained from 0 to 10 min, followed by a linear gradient to acetonitrile (45%), methanol (10%), methylene chloride (23%) and hexane (22%) from 10 to 40 min. At the end of the gradient, the column was set at the initial conditions for 20 min. Analysis of each sample was performed in duplicate. Individual carotenoids were identified by their retention time, absorption and fine spectra. The carotenoid peaks were integrated at their individual maximal wavelength, according to Khachik et al. (1992). Their quantification was carried out by comparison with external standards of lycopene, γ-carotene, δ-carotene, β-carotene, lutein, phytofluene and phytoene (R^2 in the range of 0.9923 to 0.9984). The concentration of each individual carotenoid was expressed as $µg·kg^{-1}$ of fresh weight (fw). The concentration of total carotenoids was calculated as the sum of individual compounds and also expressed as $µg·kg^{-1}$ (fw).

2.6.3. Bioaccesssibility calculation

The bioaccessibility of each individual compound was determined using equation (1). The results were expressed as percentage.

$$Bioaccessibility\ (\%) = \frac{CC_{digested}}{CC_{undigested}} x\ 100 \qquad (1)$$

where $CC_{digested}$ corresponds to the overall concentration of each carotenoid in the micellar fraction and $CC_{undigested}$ is the concentration in non-digested samples.

2.7. Statistical analysis

Statistical analyses were performed with the JMP Pro v.12.0.1 software (SAS Institute, Cary, NC, USA). Results are reported as mean ± standard deviation (n = 8). Analysis of variance (ANOVA) followed by Tukey-Kramer post hoc test was carried out in order to establish statistical differences among mean values. A correlation analysis was performed using a Pearson´s test. The significance level was set at 5%.

3. Results and discussion

3.1. Carotenoids profile of added-oil purees obtained from PEF-treated tomato fruits

The application of PEF to tomato fruits as a pre-processing treatment significantly enhanced ($p < 0.05$) the concentration of total and individual carotenoids in the subsequently obtained oil-added tomato purees (Table 2). The concentration of carotenoid compounds in the derived products was shown to be significantly influenced ($p < 0.0001$) by the electric field strength applied to tomato fruits since this is the main parameter impacting the overall energy input delivered. Nevertheless, the pulse number did not exhibit any significant ($p > 0.05$) effect on the concentration of each individual carotenoid.

Treatments conducted at 2 $kV \cdot cm^{-1}$ led to the greatest enhancement in total and individual carotenoids concentration in the oil-added purees. The application of PEF treatments delivering an specific energy input of 2.31 $kJ \cdot kg^{-1}$ (2 $kV \cdot cm^{-1}$ and 30 pulses) to whole tomatoes led to a 52% increase in the concentration of total carotenoids in the processed tomato product, reaching values of 13271 ± 265 $\mu g \cdot kg^{-1}$. At such PEF treatment conditions, the concentrations of phytoene and phytofluene in the purees were also enhanced by 178% and 131%, respectively, compared to those obtained when processing untreated tomatoes.

Table 2. *Concentration of carotenoids in oil-added purees obtained from PEF-treated tomatoes.*

Energy input (kJ · kg^{-1})	Carotenoid compounds concentration (µg·kg^{-1})															
	Phytofluene		Phytoene		Lycopene		δ-carotene		Lutein		γ-carotene		β-carotene		Total carotenoids	
Untreated	622 ± 30	e	664 ± 10	e	4000 ± 192	e	80.8 ± 1.6	f	240 ± 12	e	112 ± 11	c	3000 ± 53	bc	8718 ± 288	f
0.02	778 ± 37	d	841 ± 18	d	3920 ± 188	e	93 ± 4	e	278 ± 44	de	122 ± 14	bc	3129 ± 131	ab	9162 ± 353	f
0.06	815 ± 15	d	889 ± 21	d	4853 ± 233	de	104 ± 4	e	269 ± 14	e	125 ± 10	bc	3220 ± 71	a	10275 ± 138	de
0.09	791 ± 14	d	919 ± 47	d	4400 ± 211	de	96.1 ± 1.7	e	273 ± 5	de	137 ± 9	ab	3216 ± 39	a	9833 ± 176	e
0.14	1391 ± 71	ab	1748 ± 106	ab	5046 ± 243	b	153 ± 5	b	367 ± 25	ab	144 ± 14	a	3221 ± 95	a	12071 ± 102	c
0.38	1411 ± 90	ab	1650 ± 112	b	5960 ± 286	a	155 ± 9	ab	270 ± 31	e	78 ± 7	d	2930 ± 70	d	12341 ± 430	bc
0.5	1305 ± 68	b	1717 ± 80	ab	5240 ± 252	b	117 ± 20	d	337 ± 30	bc	111 ± 7	c	2818 ± 82	cd	12097 ± 847	bc
0.83	1143 ± 71	c	1354 ± 124	c	4560 ± 219	cd	134 ± 4	c	316 ± 22	cd	88 ± 6	d	2913 ± 118	cd	10524 ± 238	d
1.38	1381 ± 97	ab	1705 ± 88	b	5888 ± 283	a	157 ± 4	ab	386 ± 27	a	120 ± 10	bc	3158 ± 69	a	12796 ± 518	ab
2.31	1438 ± 45	a	1846 ± 55	a	6072 ± 292	a	165 ± 5	a	382 ± 38	ab	134 ± 14	ab	3233 ± 111	a	13271 ± 265	a

Values are expressed as the mean ± standard deviation (n = 8). Different letters within the same column mean significant differences ($p < 0.05$).

The biosynthesis of phytoene by the condensation of two molecules of geranyl-geranyl diphosphate is the first committed step in carotenoids biosynthetic pathway (Karppinen et al., 2016). Therefore, the increased concentration of phytoene in the oil-added tomato puree obtained from PEF-processed tomatoes suggests that the disruption of fruit tissue triggered by PEF could activate the transcription of genes encoding enzymes such as phytoene synthase (SlPSY), responsible for the activation of the carotenoids biosynthetic pathway in the fruits (Vallverdú-Queralt, Oms-Oliu, et al., 2013). An enhancement in lycopene concentration was also noticed in purees obtained from PEF-treated tomatoes, ranging from 4400 ± 211 $\mu g \cdot kg^{-1}$ to 6072 ± 292 $\mu g \cdot kg^{-1}$. The maximum lycopene concentration was attained when tomatoes were subjected to the most intense PEF treatment (2.31 $kJ \cdot kg^{-1}$), leading to a 1.5-fold increase in relation to the product prepared from untreated tomatoes. The increased concentration of lycopene correlated well with the enhancement of phytoene and phytofluene content ($p < 0.0001$; $r = 0.7612$ and $r = 0.7661$, respectively) in the oil-added purees made of PEF-processed tomatoes. This fact could be likely to be due to the activation of the carotenoid isomerase enzyme (CRTISO), which is involved in the conversion of phytoene to lycopene. This is consistent with the results reported by Vallverdú-Queralt et al. (2013) who proposed the intervention of CRTISO enzyme as responsible of the increased concentration of lycopene in tomato juice made of PEF-treated tomatoes.

After lycopene, the carotenoid biosynthetic pathway is divided in two branches. One route, ε,β–branch, produces δ-carotene and lutein. The alternative pathway, β,β–branch, leads to the synthesis of γ-carotene and β-carotene, among others, providing precursors for the synthesis of abscisic acid (ABA) (Liu, Shao, Zhang, & Wang, 2015). The concentration of these minor carotenoids present in tomato significantly increased ($p < 0.05$) in the oil-added purees obtained from tomatoes subjected to PEF treatments (Table 2). Thus, the concentration of δ-carotene in the derivative product obtained using untreated tomatoes was 80.8 ± 1.6 $\mu g \cdot kg^{-1}$, and increased by 104% when treatments delivering a specific energy input of 2.31 $kJ \cdot kg^{-1}$ (30

pulses at 2 kV·cm^{-1}) were applied. Under those treatment conditions, the concentration of β-carotene increased by 8% in comparison to the same product obtained from untreated tomatoes. On the other hand, the concentration of γ-carotene increased from 112 µg·kg^{-1} to 144 µg·kg^{-1} when treatments were conducted at 1.2 kV·cm^{-1} and 5 pulses. Lutein concentration rose by 61 % after applying 18 pulses at 2 kV·cm^{-1}. Correlation analysis displays that the concentrations of δ-carotene and lutein correlated well with the content of lycopene ($p < 0.001$; $r = 0.8136$ and $r = 0.5575$, respectively), whereas γ-carotene and β-carotene were not found to be significantly correlated ($p > 0.05$) with the increased amount of lycopene in the oil-added purees made of PEF-treated tomatoes. These results suggest that PEF treatments could activate the transcription of genes encoding enzymes such as lycopene ε-cyclase (LCY - E) to a greater extent than those encoding lycopene β-cyclase (LCY - B). It has already been reported that any signal, including abiotic stress factors, could activate and regulate a specific carotenoid branch via rate-limiting enzymes in tomato fruits (Liu et al., 2015). In addition, the lesser increase in the amount of carotenoids related to β,β–branch allows hypothesising that these carotenoids could promote the production of ABA. This phytohormone is considered as a carotenoid-derived compound that is predominantly involved in abiotic stress adaptation (Liu et al., 2015; Sabbagh, Lakzayi, Keshtehgar, & Rigi, 2014). In this regard, Manzi, Lado, Rodrigo, Arbona, & Gómez-Cadenas, (2016) have previously reported the decreased pool of β, β-carotenoids together with a significant ABA accumulation when plants were subjected to stressful conditions.

Furthermore, the increased concentrations of total and individual carotenoids in oil-added tomato purees obtained from PEF-treated tomatoes could be related not only to the activation of the secondary metabolism (Galindo et al., 2009; Vallverdú-Queralt, Oms-Oliu, et al., 2013) but also to the improvement of the extraction of intracellular components as a result of the electropermeabilization of cell membranes (Guderjan, Töpfl, Angersbach, & Knorr, 2005; Luengo, Álvarez, & Raso, 2014; Zderic et al., 2013). It is well established that PEF treatments are related to selective damage of biological cell

membranes, which may produce reversible or irreversible pore formation depending on the treatment intensity (Soliva-Fortuny et al., 2009). In this regard, preliminary studies revealed a significant softening in tomato tissues when fruits were subjected to field strengths ranging from 0.4 to 2 $kV·cm^{-1}$. This phenomenon could be linked to the formation of membrane pores induced by PEF, resulting in the enhanced extraction of intracellular compounds. Similar results were obtained by Luengo et al. (2014), who found that the extraction of carotenoids from tomato peels was improved after the application of PEF treatments with an electric field strength below 5 $kV·cm^{-1}$.

3.2. Bioaccessibility of individual carotenoids of added-oil purees obtained from PEF-treated tomato fruits

The bioaccessibility of total and individual carotenoids of oil-added purees obtained from untreated and PEF-treated tomatoes greatly depended on the compound at stake and the PEF treatment conditions. On the one hand, carotenoids bioaccessibility widely varied depending on the individual compound analyzed, ranging from 2.4 to 43.2% (Table 3). Among all the carotenoids analysed, lycopene exhibited the lowest bioaccessibility values (2.4 ± 0.2%). In contrast, phytoene and phytofluene had the highest bioaccessibilities, exhibiting values of 43.2 ± 5.0% and 23.8 ± 3.0% respectively. This fact was already observed by Mapelli-Brahm, Corte-Real, Meléndez-Martínez, & Bohn, (2017) who concluded that not only the hydrophobicity, but also the structure and shape of the molecule, characterized by its chain length and number of conjugated double bonds, play an important role in the bioaccessibility of carotenoids.

On the other hand, with regard to the effect of PEF on the bioaccessibility of carotenoids, the application of these treatments as a pre-processing treatment of whole tomatoes significantly ($p < 0.05$) enhanced the bioaccessible fraction of most of the individual carotenoids in the subsequently obtained tomato product (Table 3). The statistical analysis displayed that the amount of each individual carotenoid found in the micellar fraction of the digested oil-added

purees was strongly influenced ($p < 0.0001$) by the electric field strength applied to whole tomatoes. Nevertheless, the number of pulses did not appear to exert a significant effect on the amount of carotenoids released from the tomato matrix. The maximum enhancement (1.37-fold increase) in total carotenoids bioaccessibility was attained in oil-added purees made of tomatoes treated with 5 pulses at 2 $kV \cdot cm^{-1}$. These treatment conditions also led to maximal increases in the bioaccessibility of δ-carotene (2%), β-carotene (53%), lutein (125%) and γ-carotene (527%). Nevertheless, lycopene bioaccessibility of tomato purees was more enhanced (137% increase) when whole fruits were treated at 1.2 $kV \cdot cm^{-1}$ and 5 pulses (0.14 $kV \cdot kg^{-1}$). Further increase in the amount of energy delivered to tomato fruits did not lead to significant changes in the bioaccessibility of β-carotene and lutein in derived products in comparison to those obtained from untreated fruits. In addition, the bioaccessibilities of phytoene, phytofluene and δ–carotene of tomato puree generally diminished ($p < 0.05$) when fruits were subjected to PEF treatments (Table 3), thus leading to less bioaccessible values (4 – 65 % lower), in comparison to those observed in products obtained from untreated tomatoes.

Differences in the bioaccessibility of individual carotenoids in the tomato derived product as affected by the application of PEF to whole fruits could be explained by the probable competitive inhibition between carotenoids at the level of micellar incorporation. It has been reported that a high-dose intake of carotenoids could antagonize the bioaccessibility of some individual compounds (Maiani et al., 2009). In this regard, studies aimed at assessing the factors governing the transfer of carotenoids to micelles concluded that β-carotene could interfere with the absorption of lutein (Maiani et al., 2009), whereas lycopene and lutein had a significant effect on the transfer of β-carotene (Tyssandier, Lyan, & Borel, 2001). This could explain that β-carotene and lutein did not exhibit significant changes in their bioaccessibility after the application of PEF treatment delivering the highest energy inputs, although their initial concentration in the purees was significantly greater than in those obtained from untreated fruits.

Table 3. Bioaccessibility of carotenoids in oil-added purees obtained from PEF-treated tomatoes.

Energy input ($kJ \cdot kg^{-1}$)	Bioaccessibility (%)															
	Phytofluene		Phytoene		Lycopene		δ-carotene		Lutein		γ-carotene		β-carotene		Total carotenoids	
Untreated	23.8 ± 3.0	a	43.2 ± 5.0	a	4.1 ± 0.7	de	18.0 ± 1.1	a	9.5 ± 1.7	c	5.5 ± 0.3	f	14.1 ± 1.7	bcd	12.4 ± 1.1	bc
0.02	13.5 ± 3.2	cde	27.5 ± 4.8	cd	2.4 ± 0.2	f	11.4 ± 1.2	bcd	8.7 ± 0.7	cd	6.0 ± 0.7	ef	10.9 ± 0.9	e	8.8 ± 1.1	de
0.06	17.6 ± 0.8	bc	32.5 ± 5.8	bcd	2.5 ± 0.4	f	13.5 ± 2.7	b	6.5 ± 1.1	d	5.8 ± 2.0	f	11.9 ± 1.4	de	10.3 ± 2.4	bcde
0.09	2.13 ± 4.0	a	36.1 ± 3.4	b	7.5 ± 1.4	b	18.5 ± 2.3	a	14.9 ± 2.7	b	11.3 ± 1.8	bc	14.5 ± 1.5	bc	13.1 ± 2.5	b
0.14	10.4 ± 0.9	e	13.4 ± 1.6	e	9.7 ± 0.8	a	9.2 ± 0.6	d	13.6 ± 1.8	b	10.1 ± 1.7	cd	15.3 ± 0.8	b	11.9 ± 0.4	bcd
0.38	21.4 ± 3.5	ab	30.4 ± 5.9	bcd	9.5 ± 1.3	a	18.4 ± 2.5	a	21.4 ± 2.2	a	34.5 ± 1.9	a	21.6 ± 2.3	a	17.1 ± 2.5	a
0.5	10.3± 2.4	e	16.1 ± 1.7	e	3.7 ± 0.5	ef	10.2 ± 1.2	cd	6.5 ± 1.3	d	5.5 ± 0.7	f	12.7 ± 0.6	cde	8.1 ± 1.2	e
0.83	15.5 ± 2.4	cd	25.1 ± 3.1	d	5.6 ± 1.4	cd	12.0 ± 1.1	bcd	8.1 ± 1.7	cd	8.6 ± 1.5	de	14.9 ± 1.4	bc	12.0 ± 2.3	bcd
1.38	13.9 ± 2.1	cde	18.4 ± 1.8	e	7.0 ± 1.1	bc	12.7 ± 2.4	bc	10.5 ± 1.9	c	12.9 ± 2.5	b	15.0 ± 2.1	bc	11.4± 2.5	bcd
2.31	12.4 ± 0.9	de	14.9 ± 0.9	e	6.3 ± 0.9	bc	10.7 ± 1.9	bcd	8.3 ± 0.9	cd	10.6 ± 1.6	bcd	14.0 ± 1.6	bcd	9.5 ± 2.0	cde

Values are expressed as the mean ± standard deviation (n = 8). Different letters within the same column mean significant differences ($p < 0.05$).

However, as far we know, there is no literature data regarding the interaction with other carotenoid compounds, such as phytoene, phytofluene or δ–carotene. Additionally, the different behaviour of each carotenoid to incorporate into the micellar phase could be also related to the polarity of each compound, which has been stated to be the major factor affecting the solubility of carotenoids into micelles (Palmero et al., 2013). Furthermore, carotenoids could be entrapped within aggregates formed probably as a result of cell wall depolymerisation triggered by PEF. This fact could decrease the amount of carotenoids available to be dissolved into micelles, thus affecting their bioaccessibility, as previously reported by Colle et al. (2010) and Svelander et al (2011) in tomato-based products processed with high pressure homogenization (HPH).

To the best of our knowledge, this is the first study evaluating the bioaccessibility of carotenoids when PEF treatments are applied to tomato fruits. It is well known that the structure of the food matrix is one of the most important factors affecting the bioaccessibility of carotenoids (Jeffery, Holzenburg, & King, 2012; Rodríguez-Roque et al., 2015). Therefore, the data obtained in this study demonstrate that PEF would facilitate the release of carotenoid compounds, probably as a consequence of the disruption of tomato matrix and the destruction of carotenoid-protein complexes. There are several studies which demonstrate that processing operations could disrupt cell walls and favour the release of carotenoids from food matrix, thus leading to the enhancement of their bioaccessibility (Kamiloglu, Boyacioglu, & Capanoglu, 2013; Parada & Aguilera, 2007). In this regard, Rodríguez-Roque et al. (2015) reported that the application of high intensity PEF treatments to fruit juice-based beverages allowed releasing the carotenoids from the food matrix, thus improving the bioaccessibility of some of these compounds. Moreover, the mechanical disruption of the food matrix induced by PEF could enlarge the contact surface for interaction with digestive enzymes, thus favouring the release of carotenoids for incorporation into mixed micelles, as previously reported by Hedrén, Diaz, & Svanberg, (2002). Furthermore, in line with the results reported by Vallverdú-Queralt, et al. (2013), the application

of PEF treatments to whole tomatoes may promote the formation of *cis*-isomers which appeared to be more efficiently incorporated into mixed micelles than *trans*-isomers, thus resulting in an increase in their bioaccessibility (Boileau, Boileau, & Erdman, 2002).

It is worth mentioning that the concentration of carotenoids released from the food matrix into the micellar phase was significantly influenced ($p < 0.0001$) by their initial concentration in the oil-added purees, with the exception of β-carotene and γ-carotene ($p > 0.05$). Due to the number of factors influencing the micellarization of carotenoids, further investigations are required in order to gain better understanding of main factors affecting the carotenoids incorporation into mixed–micelles after applying PEF treatments to whole fresh commodities.

4. Conclusions

The application of PEF-treatments to whole tomatoes generally increased the concentration and the bioaccessible fraction of the total and individual carotenoids on the subsequently obtained oil-added puree. Carotenoid compounds in oil-added tomato puree were enhanced by increasing the electric field strength of the treatment. Thus, tomato purees obtained from PEF-treated tomatoes subjected to 2 $kV·cm^{-1}$ and 30 pulses exhibited the maximum enhancement in total and individual carotenoids. On the other hand, the concentration of total and individual carotenoids in the micellar fraction after an *in vitro* gastrointestinal digestion was increased when purees were obtained from PEF-treated tomatoes, especially in those subjected to 5 pulses at 2 $kV·cm^{-1}$. With such PEF treatments, 132%, 125%, 527%, 53% and 37% increases in the bioaccessibilities of lycopene, lutein, γ-carotene and β-carotene and total carotenoids, respectively, were attained. These findings will be useful to the tomato processing industry since they demonstrate the benefits of applying PEF to whole tomatoes as a pre-processing treatment in order to obtain tomato–based products with improved health-related properties. Further investigations are

necessary to study the potential effect of these products on human health.

5. Abbreviations

PEF, pulsed electric fields; ROS, reactive oxygen species; BHT, butylated hydroxytoluene; SSF, simulated salivary fluid; HPLC, high performance liquid chromatography; $CC_{digested}$, carotenoid concentration in the micellar fraction; $CC_{undigested}$, carotenoid concentration in non-digested samples; ANOVA, analysis of variance; SIPSY, phytoene synthase; CRTISO, carotenoid isomerase enzyme; ABA, abscisic acid; LCY-E, lycopene ε-cyclase; LCY-B, lycopene β-cyclase; HPH, high pressure homogenization.

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1. Background

Pulsed electric fields (PEF) and pulsed light (PL) treatments are non-thermal food processing technologies that have attracted growing interest as novel ways to induce stress reactions in metabolically active fruits. Plant responses induced by stress signals can be used as a stimulus to target plants for the production of secondary metabolites. Therefore, the application of PEF and PL technologies to enhance the health related properties of fruits and vegetables may arise as a promising strategy to develop healthier plant-based foods of a higher quality. In the current work, the effect of the application of PEF and PL on the carotenoids content as well as on the main physicochemical attributes of tomato fruits was evaluated in a first stage. Secondly, some factors influencing carotenoid concentrations in tomato-based products were studied. In this regard, the influence of the fruit ripeness stage, the kind of mechanical processing, the addition of oil as well as the application of PEF treatments to the intact tomato fruits before processing were assessed. In addition, the influence of these factors on the bioaccessibility of carotenoids in the obtained tomato derivatives was eventually evaluated.

2. Enhancing the carotenoids concentration in tomato fruits

2.1. Application of PEF treatments

PEF have been reported to effectively enhance the production of secondary metabolites in fruits and vegetables (Balasa & Knorr 2011; Vallverdú-Queralt et al. 2012). In this thesis, a first experimental study was carried out in order to evaluate the effect of the application of PEF treatments on the carotenoids concentration of tomato fruits after refrigeration at 4 ºC for 24 h. Different electric field strengths (0.4, 1.2 and 2 $kV \cdot cm^{-1}$) and number of pulses (5, 18 and 30 pulses), resulting in specific energy inputs ranging from 0.02 $kJ \cdot kg^{-1}$ to 2.31 $kJ \cdot kg^{-1}$, were used. Concurrently, the respiratory activity and the main physicochemical properties of raw tomatoes as affected by PEF treatments were studied. Based on the results obtained in the first experiment, two specific energy inputs were selected with the aim of

evaluating the effect of post-treatment storage conditions on the carotenoid profile and quality attributes of tomato fruits.

2.1.1. Effects on carotenoid accumulation

The application of PEF treatments enhanced the amount of total carotenoids (TCC) and lycopene (LC) in whole tomatoes stored after processing for 24 h at 4 ºC. Both TCC and LC were differently influenced by treatments of different specific energy input. However, the increase in pulse number at constant electric field strength was not found to directly correlate with the increase in the amount of carotenoids. PEF treatments conducted at 2 $kV \cdot cm^{-1}$ led to the greatest carotenoids concentration. A maximum 50% increase in TCC was observed in tomatoes subjected to treatments delivering a specific energy input of 2.31 $kJ \cdot kg^{-1}$ (2 $kV \cdot cm^{-1}$ and 30 pulses). After such treatments, LC exhibited a 53% increase in comparison to the content found in untreated tomatoes. These results are in accordance with those reported by Vallverdú-Queralt et al. (2012, 2013), who suggested that the increased concentration of carotenoids could be attributed to the activation of the carotenoid biosynthetic pathway and the enhanced extractability of these compounds from the food matrix caused by the electroporation of the cell membranes. Moreover, the application of PEF treatment also improved the lipophilic antioxidant capacity (LAC) of tomato fruits, thus leading to a 60% increase in tomatoes subjected to an energy input of 0.38 $kJ \cdot kg^{-1}$. The increase in LAC was found to significantly correlate with the accumulation of carotenoids, mainly lycopene, given that this carotenoid is the major responsible of the LAC in tomato (Cano et al. 2003).

Based on the results obtained in the first experiment, two specific energy inputs (0.02 and 0.38 $kJ \cdot kg^{-1}$) were selected with the aim of assessing the effect of post-treatment storage conditions, namely time (1, 3 and 5 days) and temperature (4, 12 and 20 ºC), on the carotenoid profile and quality attributes of tomato fruits. The carotenoid content in tomato fruits was affected by the storage time and temperature, as well as the interaction of these factors with PEF processing. Just after

PEF processing, a significant enhancement in total and individual carotenoid concentrations was observed in tomatoes subjected to treatments delivering energy inputs of 0.38 kJ·kg^{-1}. However, the application of milder treatments (0.02 kJ·kg^{-1}) did not produce instant changes in the carotenoid content. This instant increase in the amount of carotenoids in the treated fruit tissues could be attributed to the higher electroporating effect of the most intense PEF treatments, which would favor the release and extraction of carotenoids from the tomato matrix (Luengo, Álvarez, et al. 2014).

As storage progressed, the concentration of carotenoids in tomatoes was differently affected depending on the energy input delivered and the post-treatment storage temperature. In general, carotenoid concentrations were noticeably greater in tomatoes stored at 12 ºC than in those fruits stored at 4 or 20 ºC. This storage temperature has already been established as optimal to maintain a regular biochemical activity of intact tomato fruits during postharvest storage, including the carotenoids biosynthesis (Vinha et al. 2013). Therefore, a cumulative effect of PEF processing and storage at 12 ºC was observed, resulting in the greatest carotenoid concentrations in tomato fruits. A dramatic increase in total carotenoids concentration was induced in tomatoes treated with 0.38 kJ·kg^{-1}, reaching a peak value (1.7-fold increase) after 24 h, and decreasing over further storage. However, tomatoes subjected to milder treatments (0.02 kJ·kg^{-1}) exhibited the highest total carotenoids concentration after 5 days of storage at 12 ºC, which corresponds to a 1.6-fold increase in comparison to untreated tomatoes. These results indicate that the most intense PEF treatments may trigger a faster accumulation of carotenoids. Carotenoids are thought to be involved in protective functions in response to stress conditions (Ramakrishna & Ravishankar 2011). Therefore, it could be assumed that tomato metabolism could be regulated depending on the treatment intensity as a way of recovery and adaptation to PEF-induced stress.

With regard to individual carotenoids, they also exhibited their greatest concentrations in PEF-treated tomatoes stored at 12 ºC. The maximum

increase in lycopene concentration (150%) was found in tomatoes subjected to PEF treatments delivering a specific energy input of 0.02 $kJ \cdot kg^{-1}$ and subsequently stored over 5 days at 12 ºC. The fastest increase in lycopene concentration was attained in tomato fruits treated with 0.38 $kJ \cdot kg^{-1}$, hence leading to a significant rise (70%) after 1 day of storage at 12 ºC. In addition, an increase in the concentration of phytoene and phytofluene was observed in PEF- treated tomatoes. This suggests that PEF could activate some genes encoding biosynthetic enzymes, such as phytoene synthase (PSY) which is responsible of the first committed step in the carotenoid biosynthesis (Vallverdú-Queralt, Oms-Oliu, et al. 2013; Liu et al. 2015). This enhancement was especially evident in tomatoes treated with the highest PEF treatment (0.38 $kJ \cdot kg^{-1}$) and kept at 12 ºC for 3 days after processing. Moreover, the concentration of other minor carotenoids was also enhanced after PEF processing. Thus, the greatest and the fastest enhancement in lutein (238%), γ-carotene (200%), δ-carotene (13%) and β-carotene (77%) was found in tomato fruits subjected to 0.38 $kJ \cdot kg^{-1}$ and subsequently stored at 12 ºC. These compounds are biosynthesized from lycopene in presence of two enzymes: lycopene β-cyclase (LCYB) and lycopene ε-cyclase (LCYE)(Lu & Li 2008). From the results obtained in this study it might be inferred that PEF could stimulate the activity of the enzymes responsible for the bioproduction of these minor carotenoids. This is in line with the observations reported by Vallverdú-Queralt et al. (2013) in a different tomato cultivar.

Although the PEF-induced responses observed in tomatoes seem to be conditioned by multiple factors, an accurate control of treatment intensity and storage temperature is necessary for the feasible application of PEF to tomato fruits as an abiotic stressor in order to obtain derivative products with enhanced health-related properties.

2.1.2. Influence on respiratory activity

Stress responses may activate many metabolic pathways, thus causing changes in metabolism, photosynthesis and respiration rate in plants (Łukaszuk, E. & Ciereszko 2012). Some authors have previously

reported that respiration plays a special role in adaptive responses of plants against unfavourable conditions as the main source of energy for plant life activities and the crossroads of total metabolism (Rakhmankulova et al. 2003). As far we know, this is the first study aimed at evaluating the respiratory activity of tomato fruits as affected by mild PEF treatments. In this section, the PEF-induced changes in the respiratory activity as well as their relation with the above-mentioned enhancement of carotenoids in tomato fruits are discussed.

The respiratory activity of tomato fruits was significantly affected by the application of PEF treatments, leading to increased oxygen consumption (R_{O_2}) and carbon dioxide production (R_{CO_2}). Either R_{O_2} or R_{CO_2} were shown to be significantly influenced by the electric field strength, regardless the pulse number. A peak in both Ro_2 and Rco_2 was observed in tomatoes subjected to treatments delivering energy inputs of 0.38 $kJ·kg^{-1}$. In addition, a significant increase in ethylene production was noticed after the application of PEF treatments, especially in tomatoes subjected to 0.09 $kJ·kg^{-1}$ (0.4 $kV·cm^{-1}$ and 30 pulses). This burst of ethylene confirms the hypothesis suggested by Vallverdú-Queralt et al. (2012), who associated the enhancement of carotenoids with a probable promotion in ethylene production induced by PEF. It has been previously reported that ethylene is involved not only in tomato ripening, but also in the processes associated with the stress-induced response (Alexander & Grierson 2002; Cramer et al. 2011). Furthermore, the application of PEF treatments beyond specific energy inputs of 0.38 $kJ·kg^{-1}$ resulted in lower R_{O_2} and R_{CO_2} as well as in a drop in ethylene production. In concomitance, the induction of acetaldehyde synthesis was triggered. These results are likely to be related to the irreversible damage caused by PEF, which could result in a severe loss of cell viability, as previously reported by Dellarosa et al. (2016) in fresh-cut apples treated with electric field strengths ranging from 0.1 to 0.4 $kV·cm^{-1}$.

A significant correlation between total carotenoid concentrations and the respiratory rates of tomato fruits could be drawn. These results confirm the relationship between the induction of a defence response

by PEF and the activation of the secondary metabolism accounting for carotenoid biosynthesis as a way to overcome the stressful conditions elicited by the treatments.

2.1.3. Effects on quality attributes

Tomato processing is normally associated with the alteration of some quality attributes, such as colour, texture, pH and total soluble solids, among others. All these parameters are closely bound to the quality of the fruit, and consequently, to the acceptance by either consumers or food processors. Therefore, the evaluation of the main physicochemical properties of PEF-treated tomatoes as well as their relation to the above-mentioned increased concentration of carotenoids appears to be essential to provide further information about the potential use of the PEF-treated fruits. In this regard, the effect of both the PEF treatment and post-treatment storage conditions on the main physicochemical properties (colour parameters, firmness, TSS and pH) of tomato fruits was discussed in this section.

Firstly, the application of PEF treatments to whole tomatoes significantly affected the colour of the fruits. Both lightness (*L**) and redness-related values (*a*/b** ratio and hue angle) were significantly influenced by the intensity of PEF treatments as well as by post-treatment storage conditions. On the one hand, the electric field strength was the main treatment parameter affecting tomato colour. The greatest colour changes were observed in tomatoes subjected to 2 $kV \cdot cm^{-1}$ and subsequently stored at 4 ºC for 24 h, regardless the number of pulses applied. Such treatments produced a significant decrease in *L** values. This deleterious changes are likely to be a consequence of tissue electroporation, which is known to trigger the decompartmentalization of oxidative enzymes, thus allowing them to come into contact with their substrates (Asavasanti et al. 2010). In addition, a significant increase in the *a*/b** ratio was found in PEF-treated tomatoes, especially in those fruits subjected to the highest electric field strength (2 $kV \cdot cm^{-1}$). The *a*/b** ratio correlated linearly with the amount of carotenoids in tomatoes, mainly lycopene. This

correlation connected the reddening of tomato with the PEF-induced accumulation of carotenoids, particularly lycopene. On the other hand, post-treatment storage conditions significantly affected the superficial colour of PEF-treated tomatoes. In this regard, the fruits subjected to treatments with an energy input of 0.02 $kJ \cdot kg^{-1}$ exhibited a progressive loss of lightness (*L**) throughout storage, whereas tomatoes treated with a more intense energy input of 0.38 $kJ \cdot kg^{-1}$ showed a dramatic decrease in *L** values immediately after PEF processing and then remained almost unchanged throughout storage. Hue angle (*hº*), which is usually associated to changes in the red colour of tomatoes, was significantly affected by PEF processing and post-treatment storage. The *hº* values of untreated and PEF-treated tomatoes significantly decreased during storage as a result of the accumulation of carotenoids. This trend was especially evident in those tomatoes stored at 20 ºC, whose colour development occurred more swiftly. However, at such storage temperature, tomatoes treated with 0.38 $kJ \cdot kg^{-1}$ exhibited significantly higher values of *hº* through the storage period than untreated tomatoes. The delay in the reddening of tomatoes observed under these conditions suggests that the convergence of the most intense PEF treatments and the highest storage temperatures had a cumulative effect, thus leading to a rapid loss of viability of cells and affecting the normal development of tomato colour. Furthermore, refrigerated storage (4 ºC) slowed down the decrease of lightness as well as the development of red chromatic values. This is consistent with the results reported by Žnidarčič & Požrl (2006), who observed that postharvest storage temperature had a significant effect on colour of intact tomatoes. As they reported, high storage temperature could increase the enzymatic activity involved in both the biosynthesis of carotenoids and the oxidation of substrates, thus resulting in greater colour changes.

Secondly, the application of PEF treatments resulted in tomato softening and loss of turgor. Firmness was strongly influenced by both PEF treatment and post-treatment storage conditions. On the one hand, the electric field strength and number of pulses were important processing parameters affecting the firmness of tomato, which sharply

decreased even when low energy treatments were applied. Tomato fruits subjected to the highest electric field strength (2 $kV \cdot cm^{-1}$) exhibited the greatest loss of firmness. The maximum firmness modification was noticed after applying treatments delivering a specific energy input of 2.31 $kJ \cdot kg^{-1}$ (2 $kV \cdot cm^{-1}$ and 30 pulses), thus decreasing by 80% in comparison to untreated tomatoes. The impact of PEF treatments on the texture and structure integrity of plant tissues is well described in literature (Toepfl et al. 2005). Textural changes, such as tissue softening for apple, potato and carrot by PEF have previously been reported by Lebovka et al. (2004) for electric field strengths ranging from 0.56 to 1.1 $kV \cdot cm^{-1}$. The softening of PEF-treated tomatoes significantly correlated with the increased concentration of carotenoids. Carotenoids in tomato accumulate in the membrane of plastids, as carotenoid-protein complexes in chloroplasts or in crystalline form inside chromoplasts (Parada & Aguilera 2007). Therefore, PEF is thought to induce several changes in membrane structure, promoting the extraction of these compounds from tomato tissues, which is consistent with the results reported for other plant tissues (Luengo, Álvarez, et al. 2014; Gachovska et al. 2013). On the other hand, post-treatment storage conditions significantly affected the firmness of both untreated and PEF-treated tomatoes. Firmness of tomatoes subjected to 0.02 $kJ \cdot kg^{-1}$ progressively decreased throughout storage and was not significantly different when compared to untreated tomatoes. Firmness decay was especially evident in tomatoes stored at 20 ºC, whereas storage at lower temperatures (4 or 12 ºC) scarcely impacted the fruit firmness. Thus, tomatoes stored at 20 ºC during 5 days exhibited 10-40% lower firmness values than those stored at chilling temperatures (4 ºC). Softening of tomato during storage is attributed to the extensive depolymerisation of cell wall pectins by polygalacturonase and pectin methylesterase (Steele et al. 1997). It is known that storage temperature significantly affects the firmness of tomato fruits, since enzymatic activity is temperature dependent (Lana et al. 2005; Tadesse et al. 2015). In contrast, tomato fruits subjected to the most intense PEF treatments (0.38 $kJ \cdot kg^{-1}$) instantly lost a 44% of their initial firmness and then remained without major changes during the storage period, regardless the storage

temperature. As previously discussed, this instant softening could be attributed to severe structural injuries caused by PEF. It seems that treatments conducted at 2 kV·cm^{-1} could exceed the critical threshold of irreversible electroporation of membranes leading to a loss of cell viability, which is in agreement with literature data (Soliva-Fortuny et al. 2009).

As well, the application of PEF treatments with different electric field strengths and pulse number significantly affected the natural pH of tomato fruits. PEF treatments delivering energy inputs beyond 0.09 kJ·kg^{-1} caused a significant increase in the pH values of tomatoes in comparison to those found in untreated fruits. Maximal pH values (4.7 ± 0.1) were attained after applying PEF treatments conducted at 2 kV·cm^{-1} and 5 pulses. Such treatment conditions also lead to a peak in the respiratory activity of tomatoes (as described in section 2.1.2.). This fact suggests that the increase pH values may be related to the acceleration of tomato respiration, which uses organic acids as substrates. As far we are concerned, this is the first study aimed at evaluating the effect of PEF in pH of whole fruits and thus results cannot be compared to those reported by other authors. However, previous studies add support to the idea that pH is involved in the response to stressful conditions, as cytosolic ions have been reported to act as secondary messengers in the elicitor-induced stress response in plants (Kader & Lindberg 2010). Concerning the evaluation of post-treatment storage conditions, it was shown that the assessed PEF treatments (0.02 and 0.38 kJ·kg^{-1}) caused an instant increase in pH values of tomatoes, which could probably act as a stress signal induced by PEF in tomato fruits. The pH values of both untreated and PEF-treated tomatoes exhibited a progressive increase throughout storage. This fact may be linked to the loss of organic acids during tomato ripening, as previously reported by Anthon et al. (2011). The application of a PEF treatment with an energy input of 0.02 kJ·kg^{-1} did not cause significant changes in the pH of tomato fruits during storage when compared to untreated tomatoes, regardless the storage temperature. In contrast, treatments delivering energy inputs of 0.38 kJ·kg^{-1} led to higher pH values than those observed in untreated tomatoes, which

could be attributed to a greater rate of consumption of organic acids induced by intense PEF treatment conditions. Moreover, storage at 20 ºC led to a faster and greater rise in pH values of tomatoes compared to those found in fruits stored at either 4 or 12 ºC. This is consistent with the results obtained by Vinha et al. (2013) who concluded that the increase in pH in tomato fruits during postharvest storage was more pronounced at higher storage temperatures.

Ultimately, PEF treatments had also a significant influence on TSS values of tomato. The most intense PEF treatment conditions (2 $kV\cdot cm^{-1}$ and 30 pulses) led to a noticeable increase in TSS of tomato fruits after 24 h of storage at 4 ºC, reaching TSS values of 5.7 ± 0.9. In contrast, tomatoes subjected to PEF treatments delivering lower energy doses did not exhibit significant changes in TSS. Some authors have hypothesized that pore formation induced by PEF may lead to a osmotic imbalance in the plant cells (Toepfl et al. 2005). Therefore, the accumulation of sugars in PEF-treated tomatoes may play a role in osmoregulation as a strategy of tomato to restore homeostatic conditions of cells (Galindo et al. 2009). Furthermore, post-treatment storage conditions were found to affect the TSS of PEF-treated tomatoes. TSS were affected just after PEF processing. However, as storage progressed, TSS were differently modified depending on the PEF treatment intensity and the storage temperature. A maximum increase of 37% in TSS was found in those fruits subjected to PEF treatments delivering specific energy inputs of 0.38 $kJ\cdot kg^{-1}$ and subsequently stored at 12 ºC for 3 days. Meanwhile, TSS in tomatoes subjected to milder treatment conditions (0.02 $kJ\cdot kg^{-1}$) reached the greatest values (5.95 ± 0.05) after 1 days of storage at 20 ºC. It has been widely reported that total soluble solids increase during tomato ripening (Anthon et al. 2011). Hence, the increased concentration of TSS in PEF treated tomatoes was consistent with the acceleration of the metabolic activity, which finally resulted in a faster ripening of the fruits.

2.2. Application of PL treatments to tomato fruits

The application of PL treatments has been recently suggested as a strategy to enhance the bioactive compounds of some fruits and vegetables (Lopes et al. 2016). However, the effect of PL processing on the carotenoid content as well as on the main quality attributes of tomato fruits has been scarcely studied.

In this Doctoral Thesis, a first experimental setup was carried out to assess the influence of the pulse light energy doses on the amount of carotenoids and the most important physicochemical properties of tomato fruits throughout 15 days of storage at 12 ºC. From the results obtained in this experiment, a specific energy dose was selected (10 J·cm^{-2}) with the aim of evaluating the effect of postharvest PL treatments of different spectral range on the carotenoids concentration as well as on the main quality attributes of tomato fruits over storage at 12 ºC.

2.2.1. Influence on carotenoids content

The carotenoids content of tomato fruits was differently influenced depending on the energy dose and the wavelength range of the PL treatments. Immediately after PL treatments, total carotenoids and lycopene concentration found in tomatoes did not significantly differ from those observed in untreated fruits. However, as the storage progressed, PL-treated tomatoes exhibited greater accumulation of carotenoids than that observed in control fruits. The first experimental approach allowed concluding that PL treatments delivering energy doses of 10 J·cm^{-2} produced the greatest increases in total carotenoids (30%) and lycopene (33%) concentrations. Therefore, this fluence was selected to assess the effect of the pulsed light spectral range on the carotenoids content of tomato fruits. It is worth mentioning that this energy dose (10 J·cm^{-2}) is lower than the maximal cumulative dose approved by FDA for the treatment of food, which is established at 12 J·cm^{-2} (FDA 2016).

The application of PL treatments of different spectral distribution significantly affected both total carotenoid and lycopene contents in tomato fruits during post-treatment storage. Untreated tomatoes exhibited a continuous increase in carotenoid concentrations during storage. In contrast, PL-treated tomatoes exhibited a sharp increase in the content of total carotenoids (8-31%) and lycopene (13-35%) between days 1 and 5, regardless the spectral range applied. Subsequently, the concentration of both total carotenoids and lycopene remained almost unchanged through storage, reaching similar values than those found in untreated fruits at day 10. These results indicate that the application of PL treatments accelerated the biosynthesis of carotenoids. The increased concentration of total carotenoids and lycopene was differently affected depending on the spectral distribution of the PL treatments. Thus, the efficiency of the emitted spectrum wavelengths increased as follows: broad ultraviolet (UV) + visible (VIS) + near infrared (NIR) wavelengths < wide spectrum light without the UV-C range < VIS + NIR. Thus, tomatoes subjected to PL treatments delivering wavelengths ranging from 400 to 1100 nm (VIS +NIR) exhibited the greatest enhancement in total carotenoids (31%) and lycopene (35%) concentrations after 5 days of storage at 12 ºC in comparison to untreated tomatoes. However, those treatments applying light containing UV wavelengths did not lead to a significantly different accumulation of carotenoids during storage in comparison to that observed in untreated tomatoes. Furthermore, an inverse correlation between carotenoids and chlorophylls was found in PL-treated tomatoes, which strengthens the idea that PL treatments accelerated the transition from chloroplasts to chromoplasts, which has been reported to occur during tomato ripening (Pataro et al. 2015).

As far we know, no information is available in literature regarding the influence of pulse light dose spectral range on the accumulation of carotenoids. Nevertheless, some authors reported that carotenoids biosynthesis in tomato fruits can be affected by environmental factors, including radiation intensity (Liu et al. 2015). According to Gautier et al. (2008), carotenoids content of tomato fruit depends on the light intercepted by the fruit itself. It has been demonstrated that red light

could activate the biosynthetic pathway of carotenoids by modulating phytoene synthase activity, which is the first committed step in carotenogenesis (Schofield & Paliyath 2005). Other authors reported that UV-light exposure accounts for the formation of free radicals which may induce carotenoids degradation (Soliva-Fortuny & Martín-Belloso 2016). In addition, Lu et al. (2016) and Tiecher et al. (2013) noted that UV-C light could delay the accumulation of carotenoids in tomato during off-vine ripening. Therefore, these facts can probably counteract the beneficial effect of the VIS-NIR fraction in those PL treatments containing UV fractions, leading to a lower accumulation of carotenoids.

2.2.2. Effect on quality attributes

Total light energy dose delivered to tomato fruit did not significantly affect the colour (lightness and hue angle), pH and TSS of the fruits. Thus, no significant differences were found in these parameters between untreated and PL-treated tomatoes stored during 15 days at 12 ºC. Our results are in accordance with those reported by Pataro et al. (2015), who observed that pulsed light treatments had not a significant influence on these quality attributes of tomato fruits. With regard to firmness, only tomatoes subjected to the most intense PL treatment (25 $J\cdot cm^{-2}$) exhibited significant tissue softening just after processing, probably due to the photophysical effects caused by sample heating under the use of high fluencies (Oms-Oliu, Aguiló-Aguayo, et al. 2010). Nevertheless, despite the loss of firmness observed over storage, no significant differences were found between untreated tomatoes and those subjected to any of the PL-treatments assessed. Similarly, neither Aguiló-Aguayo et al. (2013) nor Liu et al. (2009) observed significant differences in tomato firmness after the application of PL, UV-C, red light or sun light treatments.

With regard to the application of PL treatments of different spectral range, doses of wide spectrum light (broad ultraviolet (UV) + visible (VIS) + near infrared (NIR) wavelengths), wide spectrum light without the UV-C range, and VIS + NIR light were compared. The application of

these treatments was not shown to have a significant effect on the main physicochemical properties of tomato over storage at 12 ºC. Thus, PL-treated tomatoes did not significantly differ in surface colour, pH, TSS and firmness from untreated fruits throughout post-treatment storage. Other authors have previously reported that UV-C light or PL treatments (whole spectrum) did not promote appreciable changes in colour, firmness, pH (Pataro et al. 2015; Aguiló-Aguayo et al. 2013) and TSS (Liu et al. 2009) of tomato fruits. Nevertheless, as far as we are concerned, this is the first work assessing the effect of PL treatments of different light spectral ranges on the quality attributes of tomato fruits. Our results provide evidences that PL treatments, especially those composed of VIS and NIR wavelengths, have the potential to induce a faster accumulation of carotenoids in tomatoes without causing significant changes in their main quality attributes during post-treatment storage.

3. Enhancing the concentration of carotenoids in tomato-based products

During last years, the development of new processing techniques that allow providing products with enhanced health-related properties is arousing increasing interest. One of the major objectives of this thesis was to obtain tomato-based products with enhanced concentration of carotenoids. In this section, the carotenoid concentration of tomato-based products as affected by different factors is discussed. Particularly, the influence of fruit ripeness stage (mature-green, pink and red-ripe), mechanical processing (cutting and grinding), oil addition (coconut, sunflower, and olive oils) and the application of PEF treatments to the intact tomato fruits was evaluated.

3.1. Influence of fruit ripeness stage

Carotenoid concentration in tomato-based products was strongly influenced by the ripeness stage of the intact fruits. Total carotenoid contents in tomato derivatives processed at green-mature stage were very low, ranging from 0.53 to 1.3 mg·kg^{-1}, depending on the kind of

processed product. As tomato fruits ripened, total carotenoids content in tomato-based products increased by 10-fold, reaching its maximum concentration in those obtained from red-ripe fruits. Lycopene contents in tomato derivatives increased about 40-fold over ripening, thus accounting for 52-57% of the total carotenoid contents in products obtained from fruits processed at fully-ripe stage (tomato cv. Raf). It is well established that tomato ripening involves several biochemical changes including chlorophyll degradation and synthesis and accumulation of carotenoids, mainly lycopene (Ilahy et al. 2011). In this regard, Hdider et al. (2013) found that lycopene concentration increased in proportion to the advanced ripeness of tomatoes, which is in accordance with our results. In addition, other authors have reported a marked increase in other minor carotenoids, such as β-carotene, γ-carotene, phytoene and phytofluene, which may contribute to the increased concentration of total carotenoids during tomato ripening (Raffo et al. 2002).

3.2. Influence of mechanical processing

Two tomato derivatives, cubes and puree, were obtained in order to evaluate the effect of mechanical processing on the content of total carotenoids and lycopene. Mechanical operations had a significant influence on the final concentration of carotenoids in tomato derivatives. The disruption of tomato tissues by grinding led to a noticeable decrease in total carotenoids (36-59%) and lycopene (40-46%) contents in comparison to those found in tomato cubes. In line with our results, some authors have previously reported a decreased amount of carotenoids during mechanical processing as a consequence of degradative processes (Martínez-Hernández et al. 2015). Since carotenoids are rich in conjugated double bonds, their degradation via isomerization or oxidation can occur in the presence of light, oxygen, heat, enzymes and peroxides (Rodriguez-Amaya 1999; Carbonell-Capella et al. 2014). Particle size reduction may increase the contact of carotenoids with pro-oxidant conditions, such as light and oxygen, thus accelerating their degradation. In addition, tissue disruption is likely to

induce an increase in lipoxygenase activity, which, in turn, can promote the oxidation of carotenoids (Martínez-Hernández et al. 2015).

3.3. Influence of oil addition

The addition of different types of dietary oils (coconut, olive, and sunflower oil) characterized by their different fatty acid composition, during tomato grinding influenced the total carotenoids concentration in the derived products. Tomato purees made of red-ripe fruits in the presence of oil exhibited lower carotenoid degradation during grinding (11 - 24%) than those without added oil (46%). Therefore, the presence of oil seemed to play a protective role against carotenoid degradation. Furthermore, the type of oil also influenced carotenoids degradation. In this regard, the addition of oils mainly composed by unsaturated fatty acids (olive and sunflower) led to a greater degradation of carotenoids (25%) in comparison to the addition of oils rich in saturated fatty acids (coconut) (11%). The oxidative stability of oils depends on the degree of unsaturation of the fatty acids chains (Ines J P Colle et al. 2013). More unsaturated oils exhibited lower stability to lipid oxidation, thus increasing the potential for the production of radical species that may react with carotenoids (Boon et al. 2010).

3.4. Effects of PEF application to intact tomato fruits

Based on the results obtained in previous experiments, the application of PEF to red-ripe tomato fruits was proposed as a pre-processing treatment in order to obtain tomato purees with added olive oil with high health-related properties. In this section, it is discussed whether the above-mentioned increased concentration of carotenoids in PEF-treated tomato fruits (section 2.1.1.) could provide greater amount of these health-related compounds in the subsequently obtained oil-added tomato purees.

The application of PEF to tomato fruits as a pre-processing treatment significantly enhanced the concentration of total and individual carotenoids in the subsequently obtained oil-added puree. Carotenoid

concentrations were found to be significantly influenced by the specific energy input applied to tomato fruits. The electric field strength was the main PEF treatment parameter affecting both total and individual carotenoid contents, regardless the pulse number. The application of PEF treatments delivering a specific energy input of 2.31 $kJ \cdot kg^{-1}$ (2 $kV \cdot cm^{-1}$ and 30 pulses) to tomato fruits led to a maximum 52% increase in total carotenoids concentration in the oil-added tomato puree obtained 24 h after the application of PEF. Similarly, the concentration of lycopene was found to be enhanced after the PEF treatment, reaching a 1.51-fold increase in purees made of tomatoes treated with 30 pulses at 2 $kV \cdot cm^{-1}$. Tomatoes subjected to these PEF treatment conditions, also exhibited a significant enhancement in other minor carotenoids in the subsequently oil-added tomato purees. The concentrations of phytoene and phytofluene, which are lycopene precursors, were enhanced by 178 and 131%, respectively. In addition, a maximum increase in the concentration of β-carotene (8%), δ-carotene (104%), γ-carotene (20%) and lutein (59%) was found in the oil-added purees obtained from tomatoes treated at 2 $kV \cdot cm^{-1}$ and 30 pulses. These results are in agreement with those reported by Vallverdú-Queralt, et al. (2013) who observed that the application of moderate intensity PEF treatments to tomato fruits increased the content of carotenoid compounds in tomato juices.

The enhanced concentrations of carotenoid compounds in the oil-added puree might be explained by the fact that PEF could activate the transcription of genes encoding enzymes, such as phytoene synthase, carotenoid isomerase, lycopene β-cyclase and lycopene ε-cyclase in tomato fruits, which are responsible for the synthesis of phytoene, lycopene, β-carotene and δ-carotene, respectively (Vallverdú-Queralt, Oms-Oliu, et al. 2013; Liu et al. 2015) as previously discussed in section 2.1.1. Therefore, PEF may stimulate the induction of secondary metabolism, thus leading to the accumulation of carotenoids in tomato fruits and in the subsequently obtained purees. In addition, the increased concentration of total and individual carotenoids in the tomato purees was likely due to the improvement on the extraction of these compounds from tomato matrix as a result of the PEF-induced

electroporation effect. According to the results obtained in our previous studies, tomato tissues exhibited evident signs of softening after applying PEF treatments conducted at electric field strengths ranging from 0.4 to 2 kV·cm^{-1}, which may be plausibly related to the formation of pores in the cells membranes (section 2.1.3). This effect may favour the release of intracellular compounds, as previously reported by other authors in different vegetable tissues (Luengo, Álvarez, et al. 2014; Zderic et al. 2013).

4. Enhancing the bioaccessibility of carotenoids in tomato derivatives

The bioaccessible fraction of bioactive compounds has been stated to be more relevant for the nutritional value of foods than their concentration in the original food (Rodríguez-Roque 2014; Knockaert, Pulissery, et al. 2012). Hence, it is important to assess not only the amount of carotenoids in tomato fruits and its derivatives, but also their bioaccessible fraction in order to clarify the potential effect on human health.

Once the factors influencing the carotenoid content in tomato derivatives were determined, their impact on the carotenoid bioaccessibility was also evaluated. A first experiment was carried out in order to evaluate the bioaccessibility of carotenoids in 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 (coconut, sunflower, and olive). Based on the results obtained in the preliminary experiments, optimal conditions were selected in order to produce a tomato derivative from PEF-treated tomatoes with enhanced carotenoid bioaccessibility. In this regard, the bioaccessible fraction of individual carotenoids in tomato purees obtained from red-ripe fruits processed 24 h following the PEF treatment and with 5% of added olive oil was evaluated. The influence of PEF treatment parameters on the carotenoid bioaccessibility was determined.

4.1. Influence of fruit ripeness stage

The bioaccessibility of total carotenoids and lycopene in the obtained tomato derivatives was significantly influenced by the ripeness stage of tomato fruits. The amount of total carotenoids released from tomato matrix improved as the fruits ripened, ranging from undetectable values at mature-green stage to 225 ± 22 $\mu g \cdot kg^{-1}$ in tomato purees obtained from red-ripe fruits. Similar results were reported by Ornelas-Paz et al. (2008) in mango fruits, thus concluding that the amount of carotenes transferred into mixed micelles during the simulated digestion is greatly influenced by the ripening stage of the digested fruit. With regard to lycopene, its concentration reached values of 83 ± 6 $\mu g \cdot kg^{-1}$ in digested samples of tomato purees made of fruits processed at the most advanced stage of ripeness, which corresponds to bioaccessibility values of 1.84 ± 0.18%. These results are in the range of values reported in literature (Colle et al. 2012). Although lycopene is the most important carotenoid in tomato, its bioaccessibility was very low. Several authors have reported that lycopene is less efficiently transferred into micelles than other carotenoids due to its greater hydrophobicity (Ryan & Prescott 2010).

In addition, the final amount of carotenoids in the digested samples was not only affected by the initial concentration of carotenoids in tomato fruits, but also to softening phenomena occurring through tomato ripening. In this regard, Ornelas-Paz et al. (2008) previously reported that the breakdown of the cell walls by pectic enzymes during ripening might increase the accessibility of carotenoids by facilitating the mechanical and enzymatic disruption of the pulp during digestion, and consequently their incorporation into micelles.

4.2. Influence of mechanical processing

The influence of tomato particle size on the bioaccessibility of total carotenoids and lycopene was assessed. Two different types of processing were evaluated: cutting in small pieces (tomato cubes) and grinding into tomato puree. The disruption of tomato matrix by

grinding enhanced the bioaccessibility of both total carotenoids and lycopene compared to the values found in tomato cubes, thus leading to a 2.54- and 2.19-fold increase, respectively, when tomato products were obtained from tomato fruits at red-ripe stage. In concomitance with these results, other authors have previously observed that mechanical treatments may be beneficial in order to improve the bioaccessibility of carotenoids of food products (Martínez-Hernández et al. 2015; Kamiloglu et al. 2013). Some previous studies have reported that processing operations, especially those involving tissue disruption, allow favouring the release of carotenoids from tomato chromoplasts, thus leading to an increase in their bioaccessibility (Maiani et al. 2009; Parada & Aguilera 2007). In addition, the reduction of the particle size may enlarge the contact surface for interaction with digestive enzymes, thus improving the release of carotenoids from the tomato matrix (Knockaert, Lemmens, et al. 2012; Parada & Aguilera 2007). Moreover, other authors have attributed the increased bioaccessibility values after processing to the probable isomerization of carotenoids (Parada & Aguilera 2007). In this sense, tissue disruption could undergo degradation of carotenoids via isomerization (from *trans* to *cis* configuration) due to their molecular configuration, rich in conjugated double bonds (Shi & Maguer 2000). Some studies have shown that *cis*-isomers are more bioavailable than *trans*-forms, probably because they are preferentially incorporated into mixed micelles (Hedges & Lister 2005; Boileau et al. 2002).

4.3. Influence of oil addition

Tomato cubes and tomato puree without oil exhibited very low bioaccessibility values for total carotenoids, ranging from undetectable values to 2.9 ± 0.4%, depending on the tomato product and the ripeness stage of the fruits at processing. It was observed that the bioaccessibility of both total carotenoids and lycopene was significantly improved when oil was incorporated to the end products. Hence, tomato products made of red-ripe tomato fruits by adding 5% of olive oil displayed the highest total carotenoid bioaccessibility (19-29%), followed by sunflower oil (7-23%) and coconut oil (5-17%). Lycopene

bioaccessibility exhibited a similar trend to that followed by total carotenoid bioaccessibility. Thus, the bioaccessibility of lycopene in tomato products ranged from 5%, after adding coconut oil, to 27%, when olive oil was incorporated. Due to the hydrophobicity of carotenoids, once these compounds are released from tomato matrix, they have to be incorporated into mixed micelles in order to be taken up by intestinal enterocytes (Huo et al. 2007). Therefore, the addition of oil to tomato products appears to be crucial for the absorption of carotenoids, which is consistent with the literature data (Colle et al. 2012; Huo et al. 2007; Nagao et al. 2013). With regard to the type of oil, the largest increase in total carotenoids and lycopene bioaccessibilities was noticed after incorporating 5% olive oil to tomato products. This fact could be related to its fatty acid composition. According to Colle et al. (2012), long-chain polyunsaturated fatty acids, which are typically found in olive oil, led to the greatest enhancement in the carotenoid bioaccessibility. Meanwhile, oils mainly composed of medium-chain fatty acids, such as coconut oil, resulted in lower carotenoid bioaccessibility. Some authors (Colle et al. 2012; Huo et al. 2007) have reported that oils rich in medium-chain fatty acids exhibited less effective swelling of the micelles compared to those composed of long-chain fatty acids, thus resulting in lower bioaccessibility, which is in accordance with the results obtained in our study.

4.4. Effects of PEF application to intact tomato fruits

The bioaccessibility of individual carotenoids in oil-added purees obtained from PEF-treated tomatoes greatly varied depending on the carotenoid compound at stake and the specific energy input of the PEF treatments, thus ranging from 2 to 43%. In general, lycopene exhibited the lowest bioaccessibility values due to its hydrophobicity, whereas phytoene and phytofluene were the most bioaccessible carotenoid compounds. Similar results have been reported in different fruit juices by Mapelli-Brahm et al. (2017), who concluded that the bioaccessibility of each individual carotenoid is strongly related to its chemical structure (chain length and number of conjugated double bonds) and matrix distribution.

The bioaccessible fraction of individual carotenoids was significantly influenced by the electric field strength of the PEF treatments. However, it was not shown to be significantly related to pulse number. Total carotenoids bioaccessibility was enhanced by 37% in tomato purees made of fruits subjected to PEF treatments delivering an energy input of 0.38 $kJ \cdot kg^{-1}$ (2 $kV \cdot cm^{-1}$ and 5 pulses). These treatment conditions also led to a maximum increase in the bioaccessibility of lycopene (132%), δ-carotene (2.2%), lutein (125%), γ-carotene (527%) and β-carotene (53%), respectively. PEF treatments delivering energy inputs beyond 0.38 $kJ \cdot kg^{-1}$ led to bioaccessibility values for δ-carotene, β-carotene, and lutein that were similar to those of untreated samples. Moreover, the bioaccessibility of phytoene and phytofluene in purees was significantly reduced when tomato fruits were subjected to any of the studied PEF treatment conditions. These results suggest that a higher carotenoid content in tomato purees was accompanied by a lower transference of these compounds into mixed micelles, which is in agreement with Priyadarshani (2017). This effect could be due to a competitive inhibition towards the absorption mechanism of a single carotenoid at the micellar level (Maiani et al. 2009).

To the best of our knowledge, no information is available in literature regarding the bioaccessibility of carotenoids in a processed plant-based food product as affected by the application of PEF treatments to the whole fruit before processing. The enhanced bioaccessibility of both total and individual carotenoids in oil-added purees obtained from PEF-treated tomatoes could be attributed to three phenomena. On the one hand, the significant correlation found between the initial carotenoid concentrations and their amount in the digested fraction suggests that the enhanced carotenoid concentrations in purees, as a result of the activation of secondary metabolism in tomato fruits (as described in sections 2.1.1. and 3.4.), translates to a greater bioaccessible fraction of these compounds. The importance of the dosage of carotenoids on their bioaccessibility is well described in the literature (Priyadarshani 2017; Castenmiller & West 1998). On the other hand, the enhanced bioaccessible fraction of carotenoids could be attributed to the promotion of the release of carotenoids from the tomato matrix. The

structure of the food matrix, as well as the specific localization of carotenoids into the chromoplasts are the major factors affecting their bioaccessibility (Jeffery et al. 2012). Hence, the electroporation effects induced by PEF could facilitate the destruction of carotenoid-protein complexes, thus leading to a greater release of carotenoids and a higher incorporation into the micellar phase. In addition, the application of sufficiently high electric fields may result in pore formation and breakage of cell membranes, thus enlarging the contact surface for interaction with digestive enzymes. This effect may improve the release of carotenoids from the tomato matrix, as previously described for other mechanical processes in fruits and vegetable matrices (Knockaert, Lemmens, et al. 2012; Hedrén et al. 2002). Finally, according to Vallverdú-Queralt et al. (2013), PEF may induce carotenoids isomerization. As they reported, significant increases in 9-, 13- and 15-*cis*-lycone and 9-*cis*-β-carotene were found in PEF-treated tomatoes. In addition, these authors report that 9- and 13-*cis*-lycopene concentrations were also significantly higher in juices made of PEF-treated tomatoes in comparison to those obtained from untreated fruits (Vallverdú-Queralt, Odriozola-Serrano, et al. 2013). Therefore, the enhanced bioaccessibility of carotenoids in purees obtained from PEF-treated tomatoes could be partly related with the more easily taken up of *cis*-isomers by mixed micelles.

The results obtained in this work evidence that the application PEF treatments to tomato fruits allow enhancing the bioaccessibility of carotenoids of the subsequently obtained purees, thus promoting their health-related properties.

5. References

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CONCLUSIONS

From the results of this Doctoral Thesis the following conclusions were drawn:

- ✓ PEF are a feasible technology to enhance the carotenoid concentration of tomato fruits. The application of treatments conducted at 2 kV·cm^{-1} led to the greatest enhancement in the concentration of total carotenoids (50%) and lycopene (53%). The accumulation of carotenoids was accompanied by a substantial acceleration of the respiratory activity. In concomitance, increased values of pH and TSS, as well as changes in the surface colour of tomato fruits were observed. Moreover, tomato firmness was strongly affected by PEF.
- ✓ The carotenoids concentration and the physicochemical properties of PEF-treated tomatoes were affected by post-treatment storage conditions. The maximum concentration of individual carotenoids was found in PEF-treated tomatoes stored at 12 ºC. The greatest enhancements of total carotenoid (58%) and lycopene (150%) were attained in tomatoes treated with 0.02 kJ·kg^{-1} and subsequently stored during 5 days. This treatment conditions did not compromise the quality attributes of tomato fruits. In contrast, the application of PEF treatments with higher energy inputs (0.38 kJ·kg^{-1}) promoted a faster accumulation of both total and individual carotenoids, although the physicochemical properties of tomatoes were significantly influenced.
- ✓ PL treatments accelerated the accumulation of carotenoids in tomato fruits without affecting their main quality attributes. PL treatments delivering energy doses of 10 J·cm^{-2} produced the greatest increases in the concentration of both total carotenoids (30%) and lycopene (33%). On the other hand, the application of PL treatments of different spectral range differently affected the accumulation of carotenoids in tomato fruits. The greatest total carotenoids (31%) and lycopene (35%) concentrations were attained in tomato fruits subjected to a light spectral range comprised between 400 and 1100 nm (VIS + NIR wavelengths) and subsequently stored for 5 days at 12 ºC.

- ✓ The application of PEF to whole tomatoes as a pre-processing treatment enhanced the individual carotenoids concentrations of the subsequently obtained tomato product. Treatments conducted at 2 kV·cm^{-1} and 30 pulses (2.31 kJ·kg^{-1}) led to a maximum increase in total carotenoids concentration (52%) as well as in the concentration of lycopene (51%), phytoene (178%), phytofluene (131%), δ-carotene (104%) and β-carotene (8%) in the oil-added tomato purees.
- ✓ Greatest concentrations of carotenoids were found in tomato derivatives obtained from fruits at red-ripe stage, which also led to an increase in their bioaccessibilities. The type of mechanical processing also influenced the content and bioaccessible fraction of carotenoids in the derived products. Thus, despite the concentration of carotenoids in tomato purees was lower than that found in tomato cubes, their bioaccessibility was much greater (up to 154%). On the other hand, the addition of 5% of different types of oils had a protective role against carotenoid degradation during mechanical processing. Additionally the presence of oil significantly enhanced the bioaccessibility of total carotenoids and lycopene in the derived products, especially when olive oil was added, reaching up to a 21-fold increase with respect to samples without oil.
- ✓ The bioaccessibility of individual carotenoids in the oil-added tomato purees can be significantly enhanced by subjecting the raw tomato fruits to PEF treatments. The electric field strength was found to be the main treatment parameter affecting the bioaccessibility of both total and individual carotenoids. Treatments conducted at 2 kV·cm^{-1} and 5 pulses (0.38 kJ·kg^{-1}) led to a 37% increase in the bioaccessibility of total carotenoids of tomato puree. The application of such PEF treatment conditions to tomato fruits also produced the maximum enhancement on the bioaccessibility of lycopene (132%), δ-carotene (2.2%), lutein (125%), γ-carotene (527%) and β-carotene (53%) in the subsequently obtained product.

FUTURE PROSPECTS

The results obtained in this Doctoral Thesis evidence the feasibility of applying PEF and PL treatments to enhance the carotenoid concentrations of tomato fruits. Furthermore, in view of the obtained results, PEF treatments can be proposed as an innovative strategy to improve the concentration and the bioaccessible fraction of carotenoids in tomato derivatives. However, these technologies require more research in order to be implemented at industrial level.

I do believe it would be interesting to keep working on the application of PEF in order to obtain other different tomato derivatives, even other fruit-based products, with enhanced antioxidant properties. This kind of products would need to be subjected to some preservative techniques, such as thermal treatments or other non-thermal technologies, in order to extend the self-life of the product. Hence, it would be interesting to investigate whether the positive effect of this technology on the health-related compounds, could be maintained after processing. On the other hand, consumer´s acceptance of fruit-based products would need to be evaluated.

Regarding PL treatments, studies carried out in this Doctoral Thesis was only a first experimental approach in order to elucidate possible applications of this technology. Hence, it becomes necessary to continue this line of research. It would be interesting to deeply investigate the effect of PL on the carotenoid profile of tomato fruits. In addition, it could be studied if the application of PL treatments could enhance the health-related properties of tomato-based products.

Therefore, this Thesis has settled down interesting basis that leaves the door open to several future investigations.

APPENDIX

POSTER PRESENTATIONS

- González-Casado, S.; Martín-Belloso, O.; Elez-Martínez, P. and Soliva-Fortuny (2015). Bioaccesibilidad in vitro de carotenoides en tomate (var. Raf): influencia de la adición de aceite de oliva y el estado de madurez. Congreso Nacional de Alimentos (VIII CYTA-CESIA). Badajoz (Spain).
- González-Casado, S.; Martín-Belloso, O.; Elez-Martínez, P. and Soliva-Fortuny (2016). The *in vitro* bioaccessibility of carotenoids in tomato puree obtained from tomatoes treated by moderate-intensity pulsed electric field. PEF School Dublin (Ireland).
- González-Casado, S.; Martín-Belloso, O.; Elez-Martínez, P. and Soliva-Fortuny (2016). Efecto de las tecnologías no térmicas sobre la bioaccesibilidad de compuestos bioactivos en tomate. Jornadas doctorales de Jaca (Spain).
- González-Casado, S.; Martín-Belloso, O.; Elez-Martínez, P. and Soliva-Fortuny (2016). *In vitro* bioaccessibility of individual carotenoids of tomato fruit as affected by the application of pulsed electric fields. EFFoST Conference . Viena (Austria).
- González-Casado, S.; Martín-Belloso, O.; Elez-Martínez, P. and Soliva-Fortuny (2017). Effect of PEF-processing and post-treatment storage conditions on the carotenoids content of tomato fruits. IFT Conference. Las Vegas (EEUU).

ORAL COMMUNICATIONS

- González-Casado, S.; Martín-Belloso, O.; Elez-Martínez, P. and Soliva-Fortuny (2016). The in vitro bioaccessibility of carotenoids in tomato puree obtained from tomatoes treated by moderate-intensity pulsed electric field. PEF School Dublin (Ireland).
- González-Casado, S.; Martín-Belloso, O.; Elez-Martínez, P. and Soliva-Fortuny (2016). Efecto de las tecnologías no térmicas sobre la bioaccesibilidad de compuestos bioactivos en tomate. Jornadas doctorales de Jaca (Spain).

AWARDS

- The best poster award: Efecto de las tecnologías no térmicas sobre la bioaccesibilidad de compuestos bioactivos en tomate. González-Casado, S.; Martín-Belloso, O.; Elez-Martínez, P. and Soliva-Fortuny (2016). Jaca (Spain).