Improved extractability of carotenoids from tomato peels as side benefits of PEF treatment of tomato fruit for more energy-efficient steam-assisted peeling

G. Pataro[1*], D. Carullo[1], Md A. Bakar Siddique[2], M. Falcone[2], N. Palo[3], G. Ferrari[1,2]

[1] Department of Industrial Engineering, University of Salerno, via Giovanni Paolo II, 132, 84084 Fisciano (SA), Italy

[2] ProdAl Scarl — University of Salerno, via Ponte don Melillo, 84084 Fisciano (SA), Italy

[3] F.P.D s.r.l, Via delle Industrie, 1, Fisciano, (SA), Italy

Abstract

The aim of this work was to assess the potential of the implementation of Pulsed Electric Fields (PEF) treatments in combination with steam blanching of tomato fruits in tomato processing, to bring, in addition to the higher energy-efficiency of the peeling process, also advantages from the improved recovery of carotenoids from their peels. The effect on carotenoids extraction of PEF treatments ($E = 0.25 – 0.75$ kV/cm; $W_T = 1$ kJ/kg) of whole tomato fruits alone or in combination with the application of a steam blanching ($T = 50 – 70$ °C, $t = 1$ min), were investigated by solvent extraction of the tomato peels ($T = 25$ °C; $t = 4$ h). The maximum extraction yields of carotenoids were observed for the strongest electric field strengths applied (+ 180% at 0.5 kV/cm and + 221% at 0,75 kV/cm). The application of PEF at $E = 0.5$ kV/cm, in combination with a 50 °C blanching, significantly increased the carotenoids content and the antioxidant power of the extracts, also with respect to the more energy-consuming conventional steam blanching treatment at higher temperatures ($T = 70$ °C). The results of this study demonstrate the potential of PEF pre-treatment, in combination with a mild steam blanching, to be implemented in the industrial processing of tomato fruits, to achieve not only a better energy efficiency of the peeling process but also the valorization of the tomato processing by-products.

Keywords— PEF, steam blanching, extraction, tomato by-products, HPLC, carotenoids, lycopene, antioxidant activity.

1. Introduction

Tomato (*Lycopersicon eculentum L.*) is grown throughout the world with an annual production that has exceeded 170 million tons in 2014 (FAOSTAT, 2014). The majority of tomato fruits produced are consumed in the processed form such as peeled tomato (whole or diced), juices, sauce, and ketchup, whose manufacturing often requires peel removal (Rock et al., 2012).

Thus, the industrial transformation of tomatoes typically includes a peeling phase of the fruits, consisting on the use of either hot lye solutions or steam blanching, (SB), which, however, suffers from various disadvantages such as disposal of caustic, high pH waste solution, and excessive water and energy consumption (Pan et al., 2009; Rock et al., 2012).

In the frame of the “FieldFood” (635632-FieldFOOD-H2020) project, we have recently investigated the possibility of coupling a mild pre-treatment of whole tomatoes by Pulsed Electric Field (PEF) at field strength and energy input below 1 kV/cm and 1 kJ/kg, respectively, with SB, as a viable peeling treatment, as compared with a conventional peeling process [The field food project, TecnAlimentaria – Food Industry, N° 8 December 2017. http://www.tecnalimentaria.it/].

However, on the basis of the vast literature available [REF], it can be expected that PEF might have a beneficial effect also on the permeabilization of the tomato peels, enabling the recovery of valuable intracellular compounds (Barba et al., 2015). The effect of PEF pre-treatment of plant tissues is the permeabilization of the cell membranes, which can facilitate the selective recovery of intracellular compounds from the inner parts of the cells upon the application of an electric field of moderate intensity ($E<10$ kV/cm) and relatively low energy ($W_T<10$ kJ/kg) (Pataro et al., 2017).

Tomato peels, together with seeds and unused pulp, are the main by-products of tomato fruit processing, representing 2-5 % in weight of the total processed tomatoes (Knoblich et al., 2005).

The tomato peels currently find low-added value uses as animal feed and fertilizers (Knoblich et al., 2005; Strati & Oreopoulou, 2014), or are directly sent to landfill (Rossini et al., 2013). However, they are still rich in important nutrients, such as proteins, lipids, carbohydrates, and fibers, and constitute a primary source of several carotenoids (Knoblich et al., 2005; Strati & Oreopoulou, 2014).

Carotenoid componds are natural pigments, characterized by essential health-promoting properties, which are accumulated in the chloroplasts and chromoplasts of several fruits during their ripening (Pataro et al., 2015; Singh et al., 2015). Lycopene is the most abundant carotenoids in tomato processing by-products. In particular, it accumulates in the peels (Strati & Oreopoulou, 2014), which contain a concentration about five times higher than in tomato seeds (Knoblich et al., 2005) and pulp (Luengo et al., 2014).

Lycopene, along with β-carotene, is an authorized natural pigment for several types of food products (Strati & Oreopoulou, 2014). Moreover, due to its remarkable antioxidant activity, it is also widely used in skin cosmetic products for its anti-aging properties (Lenucci et al, 2015), and as food supplement or nutraceutical ingredient in the formulation of food products, because of the evidence of its action in reducing the risk of cardiovascular diseases, atherosclerosis, prostate cancer and cognitive impairment (Lin & Chen, 2003; Queralt et al., 2013; Strati & Oreopoulou, 2014; Zuorro et al., 2011).

In recent years, following the increasing awareness regarding the health benefits associated with carotenoids, their global market exhibited a tremendous growth, which is expected to reach around US$ 1.53 billion in 2021, with a compound annual growth rate (CAGR) of 3.78% between 2016 and 2021 (MarketsandMarkets, 2016). This increasing trend is also reflected by the growing number of patents deposited worldwide on the extraction processes of carotenoids from natural sources (Riggi, 2010; Strati & Oreopoulou, 2014).

Conventional extraction processes of carotenoids are usually based on the maceration of the by-products using an organic solvent (e.g., acetone, hexane, ethanol, diethyl-ether, methanol and petroleum ether) or a solvent mixture with high affinity for lipid-soluble compounds (Lin & Chen, 2003; Strati & Oreopolou, 2011a, 2011b). However, these methods are time-consuming, and often require large amounts of solvents, relatively high temperature, and may eventually lead to the degradation of thermosensitive compounds, such as carotenoids, as well as to the co-extraction of undesirable components, increasing the downstream processing costs (Luengo et al., 2014; Strati & Oreopoulou, 2014). In addition, before extraction, the by-products often require pre-treatments, such as comminution and drying, which are costly and may cause significant losses of valuable compounds (Knoblich et al., 2005; Luengo et al., 2014; Strati & Oreopoulou, 2014).

For these reasons, the application of innovative wet disruption methods, such as PEF, has been proposed as an intensification pre-treatment for extraction of valuable intracellular compounds from food residues, which is also able to prevent their degradation, reduce the energy costs, the solvent consumption and shorten the treatment time (Luengo et al., 2014).

Many investigations have proved that PEF can enhance the extraction yield of water-soluble natural pigments and antioxidant compounds such as polyphenols, flavonoids, and anthocyanins from a wide range of food processing by-products (Barba et al., 2015; Bobinaitė et al., 2015; Boussetta et al., 2012; Chemat et al., 2017; Corrales et al., 2008; Luengo et al., 2013; Parniakov et al., 2016; Pataro et al., 2017), while there are limited data about the effect of PEF on the extraction of non-polar compounds (Luengo et al., 2014; Yin et al., 2008).

In particular, to date, only the study of Luengo et al. (2014) has addressed the PEF-assisted extraction of lipid-soluble compounds, such as carotenoids, from tomato peels, which have been treated by PEF after hand peeling of fresh tomatoes.

In addition, to date, no studies have been published on the extractability of carotenoids from tomato processed by-products (peels), after steam blanching (SB) of whole tomato fruits.

Therefore, the objective of this work was to investigate the use of PEF and SB, alone and in combination, as pre-treatments of whole tomato fruits, , to elucidate the potential of achieving, in addition to the reduced energy consumption of SB treatment, also a better extractability of carotenoids with high antioxidant activity from tomato processing by-products (peels).

2. Materials & Methods

2.1. Chemicals and raw material

HPLC grade methanol and acetonitrile as well as acetone, iron (III) chloride hexahydrate ($FeCl_3 \cdot 6H_2O$), and 2,4,6-tripyridyl-s-triazine (TPTZ) were purchased from Sigma-Aldrich (Steinheim, Germany). Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) was obtained from Acros Organics (Geel, Belgium). Sodium acetate and acetic acid were purchased, respectively, from Panreac (Panreac Quimica, Barcelona, Spain) and Fisher (Fisher Scientific, Rodano, Italy).

Tomatoes of "*Pachino*" variety were purchased from a local supermarket and stored in refrigerated conditions (4±1 °C) until use, within 5 days from purchase.

2.2. PEF apparatus

PEF-assisted extraction of carotenoids from tomato peels was carried out using a laboratory scale batch system. It consisted of a high voltage pulsed power (20 kV-500 A) generator (Modulator PG, ScandiNova, Uppsala, Sweden) able to generate monopolar square wave pulses (3-25 μs, 1-450 Hz). The generator was connected by a high voltage cable to a batch parallel plate treatment chamber (Donsì et al., 2011) with an electrode area of 75 cm^2, while the distance between the electrodes could be adjusted up to 5 cm, depending on the volume of the treated sample. The actual voltage and current signals in the treatment chamber were measured using a high voltage probe (Tektronix, P6015A, Wilsonville, OR, USA) and a Rogowski coil (2-0.1 Stangenes, Inc., USA) connected to a 300 MHz digital oscilloscope (Tektronix, TDS 3034B, Wilsonville, OR, USA). The maximum electric field intensity (E, in kV/cm) and total specific energy input (W_T, in kJ/kg) were calculated as reported in Bobinaitė et al. (2015).

2.3. PEF, SB, and PEF+SB-assisted extraction

Before processing, samples of whole tomato fruits of similar color and weight were manually selected and subjected to PEF, SB or PEF+SB pre-treatments. In a first set of experiments, PEF pre-treatments alone were carried out by exposing the tomato fruits at different field strengths (E = 0.25, 0.50, and 0.75 kV/cm) at a constant total specific energy input (1 kJ/kg), frequency (10 Hz) and pulse width (20 μs). These PEF parameters were chosen on the basis of preliminary experiments to preserve the fruit integrity, and improve its peelability (The field food project, TecnAlimentaria – Food Industry, N° 8 December 2017. http://www.tecnalimentaria.it), while inducing a sufficient degree of cell membranes permeabilization of tomato peels at minimum energy consumption. In all PEF experiments, the initial temperature of the samples was 20±1 °C and no appreciable temperature increase was detected due to the low energy input delivered during the treatment.

After the electrical pre-treatment, tomato fruits were hand peeled, and square pieces (1 cm^2) were cut out of the removed peels. Approximately 1 g of tomato peels was immediately placed into a 100 mL pyrex flask where acetone was added at a constant solid to liquid ratio (1:40 g/mL). The flasks were incubated for 4 hours in a water bath set at 25 °C, under constant shaking at 160 rpm. These extraction conditions were sufficient to reach significant extraction yields of the target intracellular compounds (data not shown). Moreover, in agreement with previous works, the low extraction temperature contributes not only to limit the operation cost, but also to avoid undesirable degradation reactions of the carotenoids (Singh et al., 2015; Strati & Oreopoulou, 2011a).

Samples of identical size and shape were manually cut from the peels recovered from untreated tomato fruits, to be used as controls.

A second set of experiments investigated the effect of a pre-treatment of tomato fruits, based either on SB alone or on its combination with PEF (PEF+SB) on the extraction yield of carotenoids from tomato peels. Fresh and PEF treated tomato fruits were subjected to SB in a lab-scale steam oven (Minea, SO25P, France) for 1 min at different blanching temperatures (T_{SB} = 50, 60, and 70 °C). Afterwards, the fruits were hand peeled and subjected to the same extraction protocol described above.

The extracts from untreated and treated (PEF, SB, PEF+SB) samples were then centrifuged at 5700 × g (PK121R model, ALC International, Cologno Monzese, IT) for 10 min at 4 °C to separate the supernatant, which was then filtered through 0.45 µm syringe filters. The final extracts were then stored at -20 °C until further analysis.

2.4. Cell disintegration index

Cell disintegration index (Z_P) was used to quantify the degree of cell membrane permeabilization of tomato peel tissues induced by PEF, SB, or PEF+SB pre-treatments of whole tomato fruits before extraction. The determination of Z_P via impedance analyses was carried out according to the method described by Bobinaitė et al. (2015). Measurements of electrical complex impedance in frequency sweep (10^3 – 10^7 Hz) were carried out by loading 5 g of square pieces (1 cm^2) cut out of the peels of untreated and treated tomato fruits into the measuring cell connected to an impedance analyzer (Solartron 1260, UK). For each treatment condition investigated, the Z_P value, ranging from 0 (for intact tissue) to 1 (for fully permeabilized tissue), was calculated on the basis of the measurement of the absolute value of the complex impedance of untreated (Z_{untr}) and treated tissue (Z_{tr}) in the low (1 kHz) and high (10 MHz) frequency ranges (Donsì et al. 2010).

$$Z_P = \frac{\left|Z_{untr(1kH\ \ )}\right| - \left|Z_{tr(1kHz)}\right|}{\left|Z_{untr(1kHz)}\right| - \left|Z_{tr(1MHz)}\right|} \quad (1)$$

2.5. Determination of total carotenoids (TC) content

The total carotenoids (TC) content of tomato peels extracts from untreated and treated samples was determined according to the method described by Lichtenthaler & Wellburn (1983). The absorbance of undiluted extracts was measured at 470 nm (A_{470}), 645 nm (A_{645}), and 662 (A_{662}), nm in a V-650 UV-Vis spectrophotometer (Jasco Inc., Easton, USA). Absolute acetone was used as a blank. The total content of carotenoids, expressed in mg/100 g of fresh weight (FW) peels, was calculated from the following equations for 100% acetone:

$$\mathrm{C_a} = 11.75\ \mathrm{A_{662}} - 2.35\ \mathrm{A_{645}} \quad (2)$$

$$\mathrm{C_b} = 18.61\ \mathrm{A_{645}} - 3.96\ \mathrm{A_{662}} \quad (3)$$

$$\mathrm{C_{x+c}} = (1000\ \mathrm{A_{470}} - 2.27\ \mathrm{C_a} - 81.4\ \mathrm{C_b})/227 \quad (4)$$

where C_a is the content of chlorophyll a, C_b is the content of chlorophyll b, and C_{x+c} is the content of carotenoids.

2.6. Evaluation of ferric reducing antioxidant power (FRAP) of extracts

FRAP assay of tomato peels extracts was carried out according to the method described by Benzie & Strain (1996) with some modification. Before the measurements, 0.3 M sodium acetate buffer (pH 3.6) was prepared by dissolving 3.1 g of sodium acetate and 16 mL of acetic acid in 1000 mL of distilled water; 10 mM TPTZ solution was prepared by dissolving 0.031 g TPTZ in 10 mL of 40 mM HCl; 20 mM ferric solution was prepared by dissolving 0.054 g of $FeCl_3 \cdot 6H_2O$ in 10 mL of distilled water.

The FRAP working solution was prepared by freshly mixing 0.3 M sodium acetate buffer, 10 mM TPTZ solution, and 20 mM ferric solution at a ratio of 10:1:1 (v/v/v). For the analysis, 2.5 mL of freshly prepared FRAP working solution and 0.5 mL of undiluted extract were mixed and incubated for 10 min at ambient temperature. The change in absorbance due to the reduction of ferric-tripyridyltriazine (Fe III-TPTZ) complex by the antioxidants present in the samples was monitored at 593 nm using a V-650 UV-Vis spectrophotometer (Jasco Inc., Easton, USA). The absorptions of blank samples (applying the same analysis conditions) were tested each time before and after analysis. Trolox was used as the standard for calibration curve and the FRAP values were expressed as mmol of Trolox equivalents (mmol TE) per 100 g of FW tomato peels.

2.7 HPLC analysis of carotenoid compounds

For the identification of individual carotenoids, the tomato peel extracts of untreated and treated samples were further analyzed by high-performance liquid chromatography (HPLC).

Carotenoids were separated using a Waters 1525 series HPLC system, equipped with a Waters 2996 photodiode array detector (DAD) (Waters Corporation, USA). Analytical separation of carotenoids was carried out in a Waters Spherisorb C18 reverse phase column (5 µm ODS2, 4,6 mm x 250 mm, Water Corporation, USA). The temperature of the HPLC column was set at 30 °C. Before the injection, the tomato peels extracts were filtered through 0.20 µm filters. The mobile phase consisted of acetonitrile/methanol (30:70, v/v). The flow rate of the mobile phase through the column and the injection volume were 1.5 mL/min and 100 µL, respectively. The absorbance detection wavelength was 472 nm.

The identification of the major carotenoids in tomato peels extracts was carried out by comparing their retention times and absorption spectra with those described in the literature data (Naviglio et al., 2006).

2.8. Statistical analysis

All experiments and analysis of collected samples were performed in triplicate. The mean values and standard deviations (SD) of experimental data were calculated. Statistically significant differences ($p \leq 0.05$) between the means were evaluated using one-way analysis of variance (ANOVA), and the Tukey's test. The Pearson product-moment correlation coefficient was used to measure the strength of the linear relationship between two variables. Statistical analyses were carried out using SPSS 20 (SPSS Inc., Chicago, USA) statistical package

3. Results and discussion

3.1. Effect of PEF treatment intensity on the carotenoids content and antioxidant power of tomato peel extracts

Figure 1 shows total carotenoids (TC) content in the peel extracts of untreated (0 kV/cm) and PEF-treated tomato fruits.

The amount of TC extracted from the untreated samples was 9.26 mg/100 g FW tomato peels, which is consistent with the observation of previous authors that a substantial amount of carotenoids (in particular lycopene) are accumulated in the skins of tomato fruits (Knoblich et al., 2005; Luengo et al., 2014; Strati & Oreopoulou, 2014). Moreover, the results also highlight that acetone is a good extraction solvent, showing a certain capability to penetrate the intact plant cells of tomato peels, where carotenoids are enclosed, and to dissolve them (Luengo et al., 2014; Strati &

Oreopoulou al., 2011a; 2011b). The application of PEF pre-treatment to the tomato fruits before peeling resulted in the intensification of the extractability of carotenoids, with a significantly ($p \leq 0.05$) higher TC content extracted from the peels compared to the control samples. Moreover, when PEF intensity was increased, the extractability of carotenoid compounds increased by 44%, 144% and 189% at 0.25, 0.50 and 0.75 kV/cm, respectively, compared with the control extraction.
The permeabilization the cell membranes of the tomato peel tissues upon the exposure of the whole fruits to an external electric field was characterized by the measurement of the Z_p values of the tomato peels, evaluated via impedance measurements. The Z_p values exhibited a statistically significant increase ($p \leq 0.05$) when the field strength increased, ranging from 0.20 at 0.25 kV/cm to 0.61 and 0.66 at 0.50 and 0.75 kV/cm, respectively. Remarkably, a highly positive correlation was observed between TC content and Z_P values (Table 1), which can be explained by remarkable the reduced mass transfer resistances, due to the permeabilization the cell membranes of the tomato peel tissues, and consequent increment in the extraction yield of carotenoids (Luengo et al., 2014).
PEF-induced permeabilization of cell membranes is effective in improving pigments extractability from plant tissues, such as anthocyanins from different matrices, such as grape pomace, blueberry press cake, purple-fleshed potato, red prickly pear peels and red cabbage (Barba et al., 2015; Bobinaite et al., 2015; Corrales et al., 2008; Gaschovska et al., 2010; Koubaa et al. 2016; Pataro et al., 2017; Puertolas et al., 2013), as well as betanin from red beets (Chalermchat et al., 2004; López et al., 2009). Moreover, Luengo et al. (2014) extracted carotenoid compounds from fresh tomato peels and found that 90-μs PEF treatment at 5 kV/cm increased the extraction yields in acetone of carotenoids by 50%, as compared to a conventional solvent extraction. However, in contrast with our work, the authors applied PEF pre-treatment directly to fresh tomato peels rather than to tomato fruits, and found a lower concentration of carotenoids in the extracted solution (about 3.2 mg/100 gFW tomato peels), which could be explained by the lower Z_P values (about 0.2 at 5 kV/cm and 90 μs) detected in spite of the higher field strength applied.

A qualitative analysis of peel extracts composition was carried out via HPLC, with the resulting chromatogram profiles, detected at 470 nm, reported in Figure 2. The profiles of the extracts from untreated and PEF-treated samples were similar, suggesting that the electrical pre-treatment neither promoted the selective extraction of specific compounds nor caused their isomerization or degradation. This is in agreement with the observations reported by other authors (Luengo et al. 2013; Luengo et al., 2014; Lopez et al. 2009; Pataro et al., 2017), who found that PEF pre-treatment did not significantly alter HPLC chromatogram profiles of the compounds detected in the extracts, probably due to the relatively mild treatment intensity applied (Kahmič-Kalamiza et al. 2014).

In particular, in our experiment, the main peak is associated with all-trans lycopene, detected at an elution time of 12.65 min (Naviglio et al., 2006). These results are consistent with those obtained via spectrophotometric analyses, which showed visible spectra with a maximum absorption at the characteristic wavelength (470 nm) of lycopene (data not shown). This is perfectly coherent with the fact that lycopene represents more than 80% of the total content of carotenoids in the fully ripened tomatoes (Pataro et al., 2015).

The strong positive correlation, observed also between TC content and lycopene content in peel extracts (Table 1), further confirmed that lycopene was the most predominant carotenoid in tomato peel.

Moreover, it is worth noting that, in comparison with the control sample, the application of PEF pre-treatment caused a remarkable increment of the peak area of 52%, 192%, and 231% at 0.25, 0.50 and 0.75 kV/cm, respectively. Similar results were observed by other authors, when comparing the anthocyanin profile of control and PEF treated extract from purple-fleshed potato and blueberries (Pataro et al., 2017; Puértolas et al., 2013).

Additionally, also the antioxidant power of the carotenoids (particularly lycopene) contained in the peel extracts was assessed using the FRAP assay.

As shown in Figure 3, the extracts obtained from the peels of PEF-treated tomato fruits possessed a significantly ($p \leq 0.05$) higher antioxidant activity than the control extracts (46–189 %). The higher the field strength, the greater the antioxidant power, even though significant differences($p \leq 0.05$) were detected only between the extracts of PEF treated samples at 0.25 and 0.50 kV/cm. Moreover, as previously observed by other authors (Luengo et al. 2014), a highly positive correlation was found between TC (Figure 1), lycopene content (Figure 2) and antioxidant activity (Figure 3) of peel extracts (Table 1), which clearly indicates that the lycopene contained in the tomato peels predominantly contribute to the antioxidant activity of the extracts.

The results of this study hence suggest that the cell disintegration level (Z_p = 0.61) achieved with the intermediate PEF treatment intensity (0.5 kV/cm) resulted in the most favorable conditions to intensify the extractability of carotenoid compounds with the highest antioxidant activity. Further investigations of PEF pre-treatment in combination with SB of tomato fruits were, therefore, carried out at 0.5 kV/cm with a constant energy input of 1 kJ/kg.

3.2. Combined effect of PEF and SB pre-treatments on Z_p, carotenoids content and antioxidant power of tomato peels extracts

Steam blanching (SB) is a unit operation typically used to facilitate peel removal from tomato fruits during the manufacturing of several tomato products. Therefore, in view of the exploitation as a cheap and rich source of natural carotenoids of the large amounts of tomato processed by-products (peels) currently produced at the industrial level, the impact of SB pre-treatment on the cell structure of peel tissues and the subsequent recovery of these compounds should be evaluated. Eventually, the application of a mild cell disintegration technique such as PEF in combination with SB of tomato fruits could be used to further intensify the extractability of valuable intracellular compounds.

In this work, extracts obtained from peels of whole tomato fruits pre-treated by SB (1min) alone or by the sequence of PEF (E=0.50 kV/cm, W_T=1 kJ/kg) and SB (1 min) at different steam blanching temperature (50, 60 and 70 °C), were analyzed in order to evaluate the impact of either the single thermal treatment or the combined treatment on the extractability of carotenoid compounds with high antioxidant activity.

The results of Figure 4 show that the extraction yield of carotenoids from peels of mild SB fruits was significantly improved (60-189%), as compared with the control extraction performed from fresh tomato peels (Figure 1). However, no significant difference was detected between the TC content of the SB-treated samples at 50 and 60 °C, whereas a significant ($p \leq 0.05$) difference was observed when the blanching temperature was increased from 60 to 70 °C.

It is likely that in the blanching temperature range examined, the improved extractability of carotenoids when increasing temperature can be related to the thermal damage induced at the cuticular level (Strati & Oreopoulou, 2011a). In fact, as shown in Figure 5, the Z_P values of tomato peels obtained upon SB pre-treatment of tomato fruits at 50, 60, and 70 °C, increased to 0.2, 0.36, and 0,57, respectively, with a significant difference observed only when the temperature was increased from 50 to 70 °C. Moreover, a strong positive correlation was observed between Z_p and TC content (Table 2). To the best of our knowledge, no previous work investigated the effect of SB of tomato fruits on the extractability of carotenoids from the peel residues, while several works dealt with the effect of the extraction temperature on the recovery of carotenoids. To this purpose, for example, Strati and Oreopoulou (2011a), observed that an increase of extraction temperature from 25 to 70 °C caused an increase in the carotenoids concentration in acetone extracts from tomato peel powder, which was attributed to the destruction of the cellular structure.

In contrast, when PEF pre-treatment was applied prior to SB, the TC content rose to significantly higher values ($p \leq 0.05$) with respect to the thermally treated samples for blanching temperatures of 50 and 60 °C, while a slight but not significant increase was observed when the temperature was increased to 70 °C. No statistical difference was, instead, observed among the PEF+SB treated samples.

However, it is worth noting that the combined treatment showed an almost additive effect in the extraction yield of TC at the blanching temperature of 50 °C, whereas a slight synergistic effect was observed at 60 °C at which the maximum value of 37.9 mg/100 g FW tomato peels was obtained. Further increasing the SB temperature up to 70 °C, instead, showed a slight but not significant decrease in the amount of TC extracted, as compared with the combined treatment performed at lower temperatures. From these results, it might be concluded that the electroporation effect induced by PEF prior to the subsequent thermal treatment enables the intensified recovery of valuable compounds at lower blanching temperature, with a consequent reduction of thermal stress that could negatively affect the extraction and bioavailability of thermolabile compounds. Similarly, previously published works demonstrated that PEF permeabilization of plant tissue before extraction has the potential of decreasing the extraction temperature without affecting the extraction yield (Loginova et al., 2011; López et al., 2009; Puértolas et al., 2013).

Results of Figure 4 positively correlate with the higher values of Z_P detected when PEF was applied prior to SB treatment (Figure 5, Table 2), indicating that the combined treatment has the potential to further enhance the degree of structural damages at the cuticular level, thus facilitating the penetration capacity of the solvent and the recovery of the carotenoid compounds.

Moreover, the results of Figure 4 are consistent with the HPLC chromatogram profiles of the extracts obtained upon the application of SB (Figure 6a) alone or of PEF+SB (Figure 6b). Interestingly, it can be observed that, once again, only the peak of lycopene was identified and that no isomerization or degradation occurred upon the application of either a mild SB treatment or the combination of PEF with SB, while they increased the yield compared to the extraction from untreated fresh peels or peels obtained upon the PEF pre-treatment of tomato fruits (Figure 2). In particular, the results of Figure 6 also indicate that the combined PEF+SB treatment markedly increased the area of the lycopene peak, which rose approximately of 200 %, 220 %, and 20 % at blanching temperatures of 50 °C, 60 °C, and 70 °C, compared to the peel extracts of SB-treated tomato fruits at the same temperatures. It is likely that the moderate temperature and PEF treatment intensity used in our experiments were high enough to intensify the extractability of carotenoid compounds but sufficiently mild to induce any degradation of carotenoids. Despite our results show a slight decrease in the TC content at the highest blanching temperature, they appears to be consistent with findings of Strati and Oreopoulou (2011a), who found that the increase of extraction temperature up to 70 °C did not cause any alterations to lycopene and other carotenoids from tomato waste, while it increased the yield, compared to an extraction at 25 °C.

As expected, the greater release of carotenoids, particularly of lycopene, detected in the extracts of peels obtained after SB or PEF+SB of tomato fruits, markedly increased also the antioxidant power of the extracts, as shown in Figure 7. In particular, in comparisons to the control extracts achieved from fresh peels (Figure 3), the extracts of peels obtained from SB-treated fruits exhibited a stronger antioxidant power, which rose approximately of 183%, 187%, and 301%, when the tomato fruits were thermally-treated at 50, 60, and 70 °C, respectively.

Furthermore, the combination of PEF with SB resulted in a significantly ($p \leq 0.05$) higher antioxidant activity of the extracts, as compared with the thermally treated samples, without any statistical difference detected only at the highest blanching temperature investigated.

The observed increases in the antioxidant activity of the peel extracts detected after SB alone or in combination with PEF (PEF+SB) when increasing the blanching temperature, correlate well with the higher content of carotenoids and lycopene in the extracts, showing a stronger correlation especially for samples obtained from fruits treated by SB alone (Table 2).

4. Conclusions

The results of this study have demonstrated the efficacy of the pre-treatments of whole tomato fruits, typically applied to facilitate tomato peelability, also on the extractability of carotenoids from tomato peels. In particular, the cell disintegration induced at the cuticular level by either the electrical and/or thermal treatment improves the penetration of the solvent into the cytoplasm by reducing the mass transfer resistances of the solubilized intracellular pigments, thus intensifying the extractability of carotenoid compounds. More specifically, the application of a Pulsed Electric Field treatment (E = 0.5 kV/cm; W_T = 1 kJ/kg; T) prior to steam blanching of tomato fruits at 60 °C, which is able to ensure a good tomato peelability while reducing the energy consumption, with respect to a steam blanching pre-treatment, exhibited a synergistic effect in promoting the extraction yield of TC. HPLC analyses revealed that lycopene was the most predominant carotenoid in the peel extracts, hence determining their resulting antioxidant activity. Moreover, these analyses also showed no evidence of isomerization or degradation of lycopene upon the application of the electrical and/or thermal pre-treatment.

This work, hence, demonstrated the potential of PEF pre-treatment, in combination with a milder steam blanching, to be implemented in the industrial processing of tomato fruits, to achieve not only a better energy efficiency of the peeling process but also the valorization of the tomato processing by-products.

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Figure captions

Figure 1 Total carotenoids (TC) content of extracts obtained from peels of untreated (0 kV/cm) and PEF-treated (W_T=1 kJ/kg) whole tomato fruits at different field strengths. Different letters above the bars indicate significant differences between the mean values (p≤0.05).

Figure. 2 HPLC-DAD profiles of carotenoids at 470 nm in the extracts from peels obtained after peeling of (a) untreated, and PEF-treated (W_T=1 kJ/kg) whole tomato fruits at (b) 0.25 kV/cm, (c) 0.50 kV/cm, and (d) 0.75 kV/cm.

Figure 3 Ferric reducing antioxidant power (FRAP) of extracts obtained from peels of untreated (0 kV/cm) and PEF-treated (W_T=1 kJ/kg) whole tomato fruits at different field strengths. Different letters above the bars indicate significant differences between the mean values (p≤0.05).

Figure 4 Total carotenoids (TC) content of extracts obtained from peels of whole tomato fruits pre-treated by SB (1min) (black bars) or PEF (E=0.50 kV/cm, W_T=1 kJ/kg)+SB (1 min) (grey bars) as a function of the steam blanching temperature. Different letters above the bars indicate significant differences between the mean values (p≤0.05).

Figure 5. Cell disintegration index (Z_p) of peels obtained after peeling of whole tomato fruits pre-treated by SB (1min) (black bars) or PEF (E=0.50 kV/cm, W_T=1 kJ/kg)+SB (1 min) (grey bars) as a function of the steam blanching temperature. Different letters above the bars indicate significant differences between the mean values (p≤0.05)

Figure 6 HPLC-DAD profiles of carotenoids at 470 nm in extracts from peels of whole tomato fruits pre-treated by (a) SB (1min) (black bars) or (b) PEF (E=0.50 kV/cm, W_T=1 kJ/kg)+SB (1 min) as a function of the steam blanching temperature. T = 50 °C (red curve); T = 60 °C (green curve); T = 70 °C (blue curve).

Figure 7. Ferric reducing antioxidant power (FRAP) of extracts obtained from peels of whole tomato fruits pre-treated by SB (1min) (black bars) or PEF (E=0.50 kV/cm, W_T=1 kJ/kg)+SB (1 min) (grey bars) as a function of the steam blanching temperature. Different letters above the bars indicate significant differences between the mean values ($p \leq 0.05$).

543 **Figure 1**

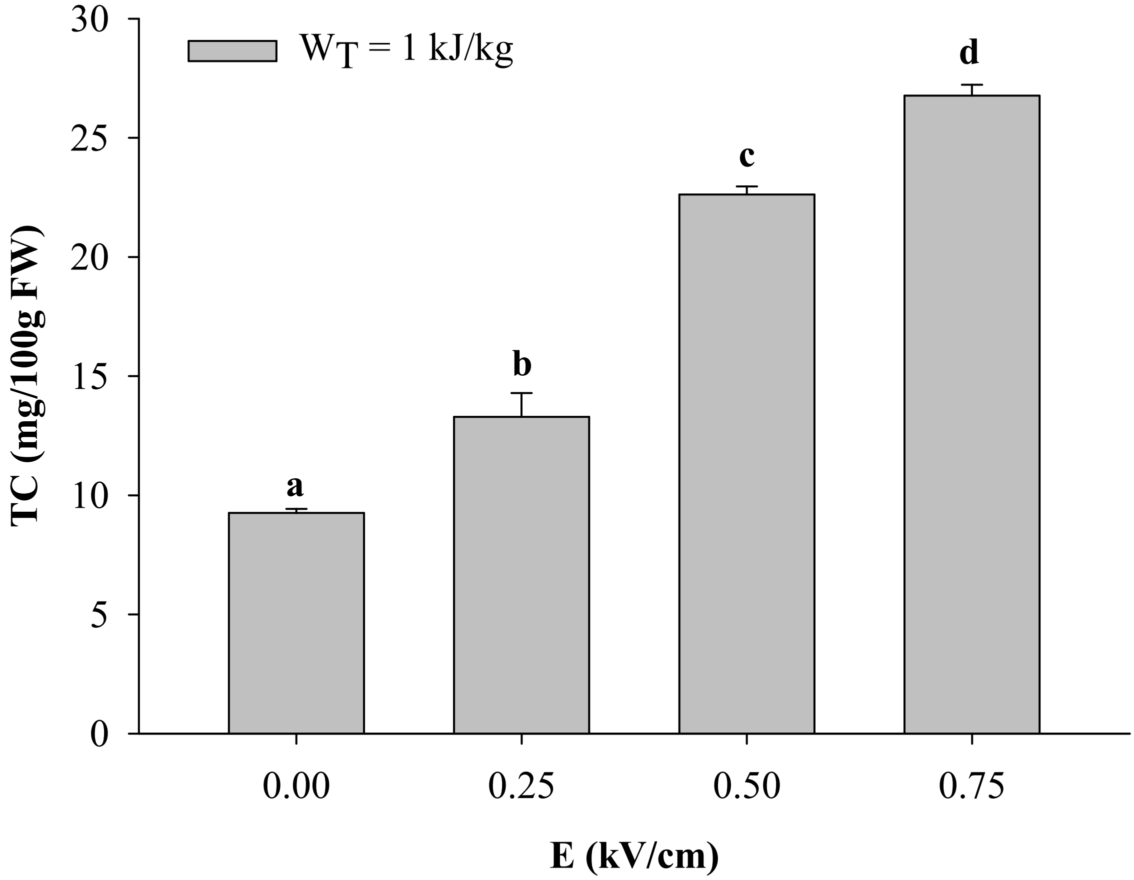

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Figure 2

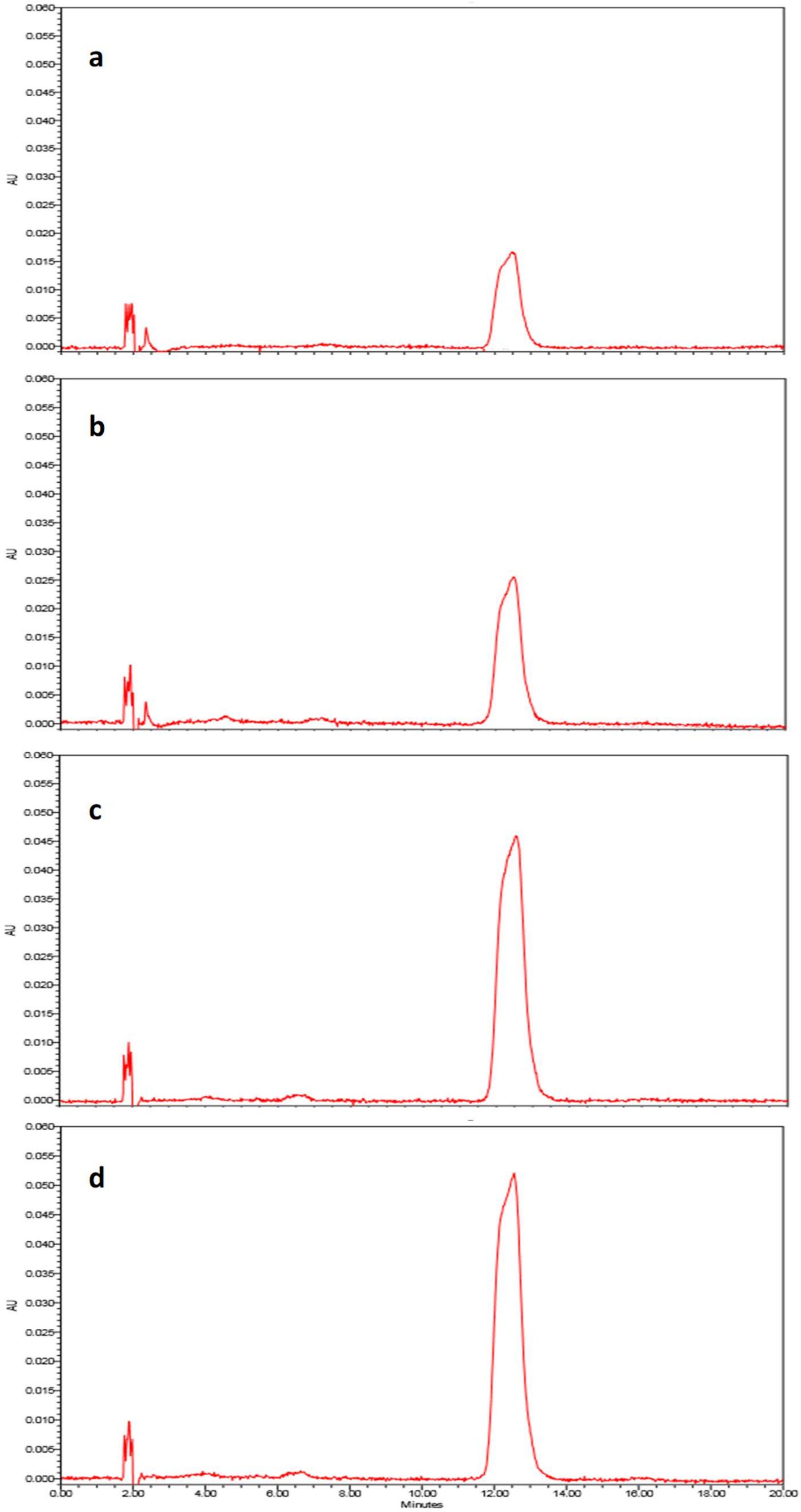
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Figure 3

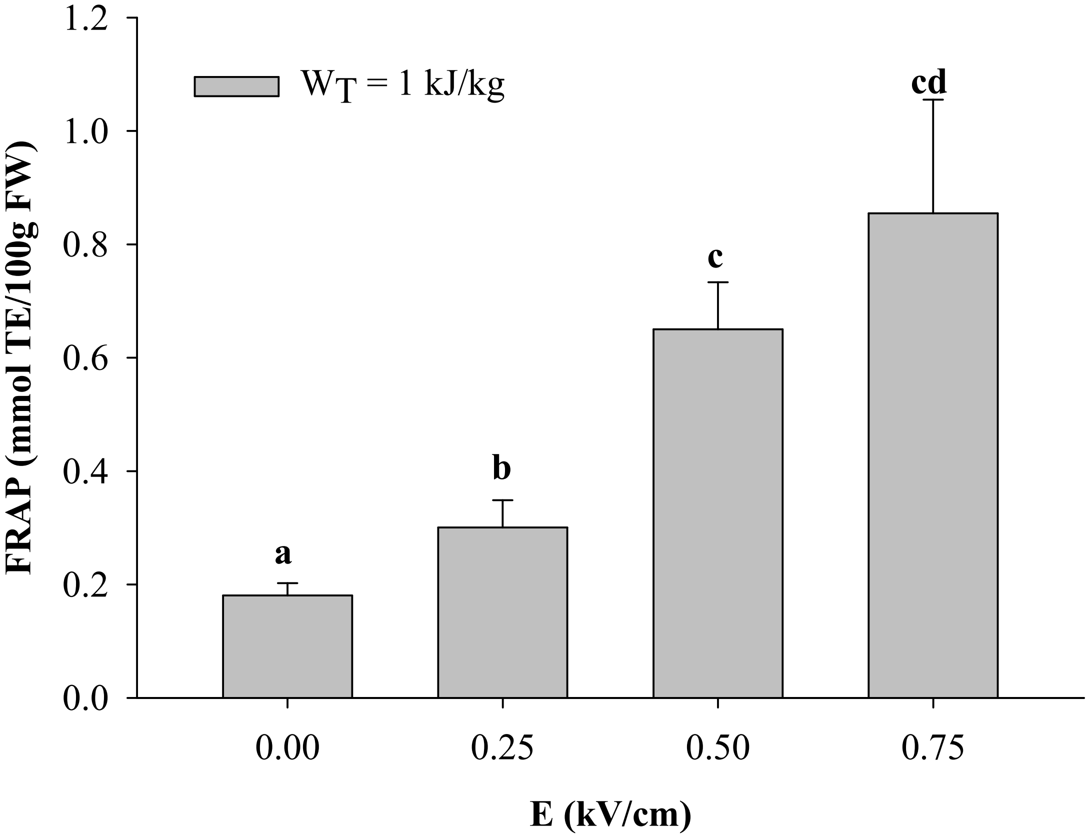


Figure 4

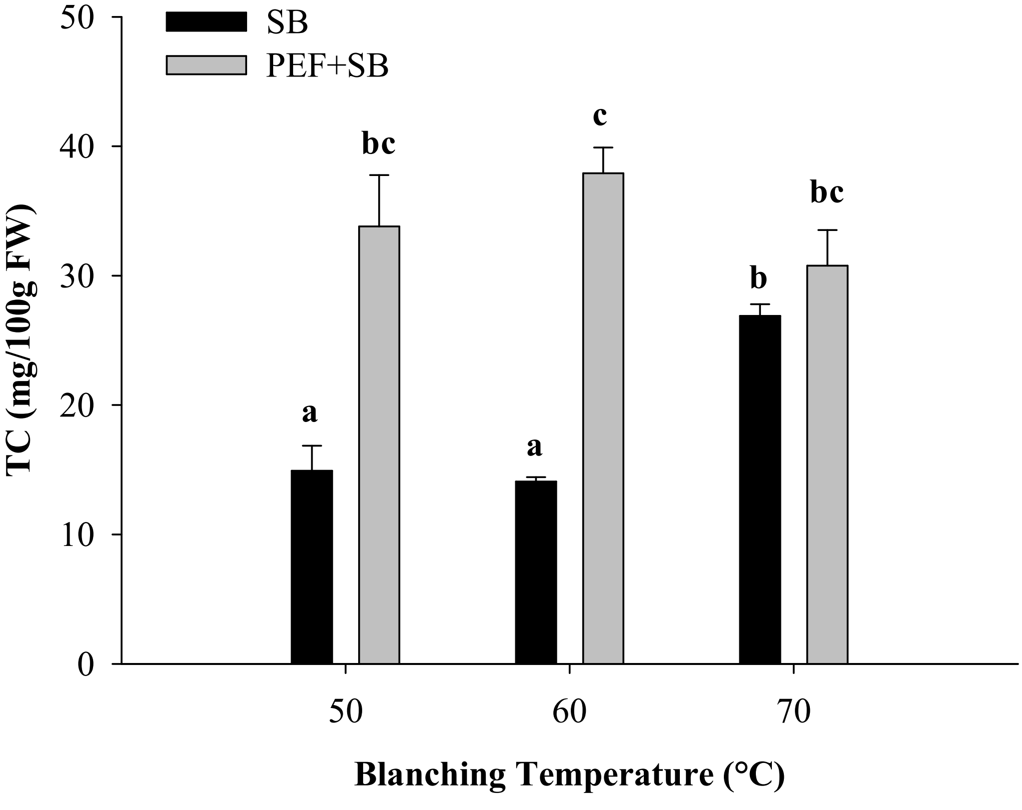
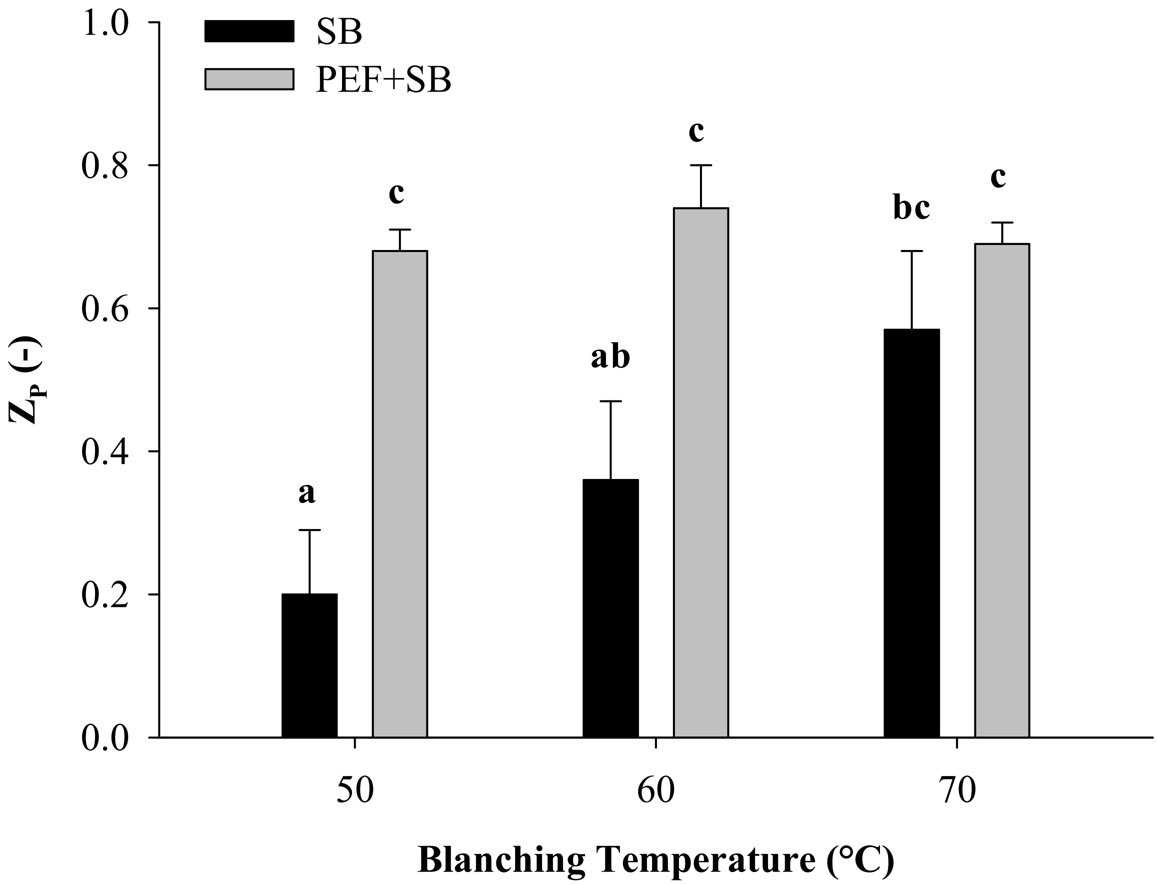


Figure 5



SB
PEF+SB
1.0
0.8
0.6
0.4
0.2
0.0
Z_P (-)
a
c
ab
c
bc
c
50
60
70
Blanching Temperature (°C)

Figure 6a

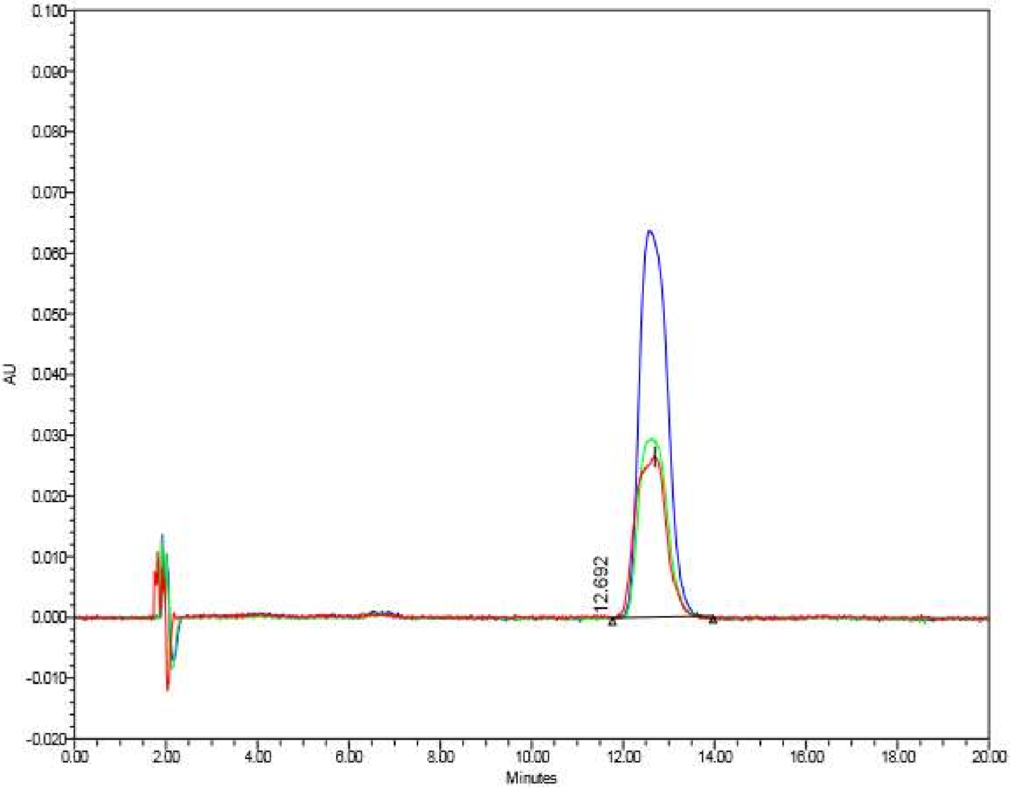


Figure 6b

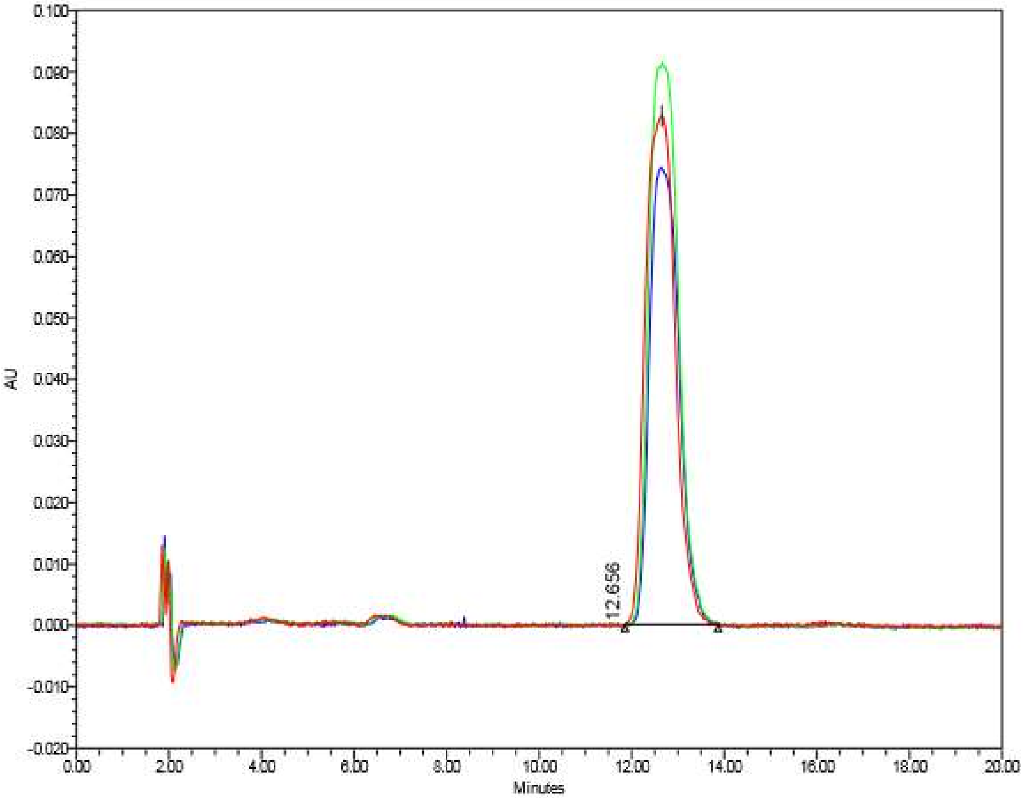


Figure 7

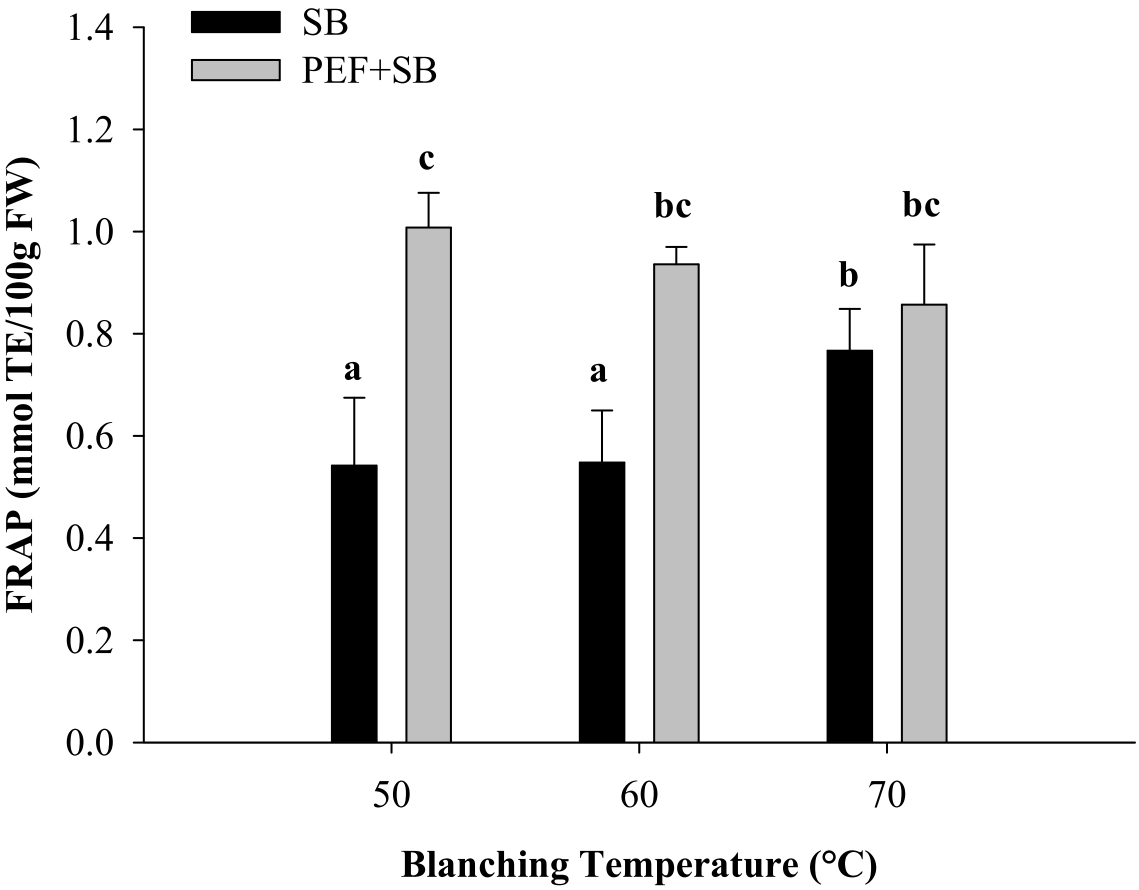

SB
PEF+SB
FRAP (mmol TE/100g FW)
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
a
c
a
bc
b
bc
50
60
70
Blanching Temperature (°C)

Table 1. Correlation coefficient among cell disintegration index (Zp) of tomato peel, and TC content, antioxidant activity (AA), and lycopene (Lyc) content of extracts from peels of untreated and PEF treated whole tomato fruits at different field strength (0.25- 0. 75 kV/cm).

Properties	Zp	TCC	AA	Lyc
Zp	-	0.978	0.961	0.994
TCC	0.978	-	0.997	0.998
AA	0.961	0.997	-	0.991
Lyc	0.994	0.998	0.991	-

Table 2. Correlation coefficient among cell disintegration index (Zp) of tomato peel, and TC content, antioxidant activity (AA), and lycopene (Lyc) content of extracts from peels obtained after peeling of whole tomato fruits pre-treated by SB (1min) or PEF (E = 0.50 kV/cm, WT = 1 kJ/kg) + SB (1 min) at different blanching temperature (50, 60, and 70 °C).

Properties	Zp	TCC(SB)	TCC (PEF-SB)	AA (SB)	AA (PEF-SB)	Lyc (SB)	Lyc (PEF + SB)
Zp	-	0.876	0.830	0.912	-0.128	0.906	0.705
TCC(SB)	0.876	-	/	0.997	/	0.998	/
TCC (PEF-SB)	0.830	/	-	/	0.447	/	0.981
AA (SB)	0.912	0.997	/	-	/	-	/
AA (PEF-SB)	-0.128	/	0.447	/	-	/	0.613
Lyc (SB)	0.906	0.998	/	-	/	-	/
Lyc (PEF + SB)	0.705	/	0.981	/	0.613	/	-