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

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Abstract

The combination of steam blanching (SB) with Pulsed Electric Fields (PEF) treatments of whole tomatoes, in addition to reducing the energy required for tomato peeling, can significantly contribute to the recovery of carotenoids from the peels.

In this work, PEF (0.25-0-75 kV/cm, 1 kJ/kg) and SB (1 min at 50-70°C), as pre-treatment prior to hand peeling, were investigated to assess their ability, separately and in combination, to induce the cell permeabilization of tomato peels, and hence to improve the carotenoids extraction in acetone (4 h at 25°C).

PEF and SB, by inducing significant damages at the cuticular level, caused the increase of the yield in total carotenoids (up to 188% for PEF and 189% for SB) and antioxidant power (up to 372% for PEF and 305% for SB) with respect to the peels from untreated tomatoes. The application of a combined treatment (PEF+SB) significantly increased the carotenoid content and the antioxidant power of the extracts, with a synergistic effect observed already at 60°C (37.9 mg/100 g fresh weight tomato peels). HPLC analyses revealed that lycopene was the main carotenoid extracted and that neither PEF nor SB caused any selective release or degradation of lycopene.

Results obtained from this study demonstrate that the integration of PEF in the processing line of tomato fruits prior to SB contributes to the valorization of 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, in 2014, has exceeded 170 million tons (FAOSTAT, 2014). The majority of tomato fruits produced are consumed in processed form, such as peeled tomato (whole or diced), juices, sauce and ketchup, whose manufacture often requires peel removal (Rock et al., 2012).

Thus, the industrial transformation of tomatoes typically includes a peeling phase of the fruits, consisting in 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).

Recently, the "FieldFood" (635632-FieldFOOD-H2020) project 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 less energy-intensive peeling treatment, as compared with a conventional peeling process (TecnAlimentaria–Food Industry, 2017).

However, as suggested by the vast literature on the topic, PEF pre-treatment might be expected to have a beneficial effect also on the permeabilization of the tomato peels, enabling the recovery of valuable intracellular compounds (Barba et al., 2015). The main effect of the application of PEF pre-treatment of plant tissues, at an electric field of moderate intensity ($E<10$ kV/cm) and relatively low energy ($W_T<10$ kJ/kg), is the permeabilization of the cell membranes, which facilitates the selective recovery of intracellular compounds from the inner parts of the cells (Barba et al., 2015; Donsì et al., 2010; 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 compounds are natural pigments, with health-beneficial properties, which are accumulated in the chloroplasts and chromoplasts of several fruits during ripening (Pataro et al., 2015; Singh et al., 2015). Lycopene is the most abundant carotenoid in tomato processing by-products. In particular, it accumulates in the peels (Strati & Oreopoulou, 2014), at concentrations 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). Because of the its activity in reducing the risk of cardiovascular diseases, atherosclerosis, prostate cancer and cognitive impairment, it is also used as food supplement or nutraceutical ingredient in the formulation of food products (Lin & Chen, 2003; Queralt et al., 2013; Strati & Oreopoulou, 2014; Zuorro et al., 2011).

For all the above reasons, in the last decade the global market of carotenoids 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 the thermosensitive compounds, such as carotenoids, as well as to the co-extraction of undesirable components, thus increasing the downstream processing costs (Luengo et al., 2014; Strati & Oreopoulou, 2014). In addition, before extraction, the by-products often require a pre-treatment, mainly comminution and drying, which is costly and may cause significant losses of valuable compounds (Knoblich et al., 2005; Luengo et al., 2014; Strati & Oreopoulou, 2014).

Therefore, the implementation of an innovative wet disruption method, such as PEF, has been proposed as an intensification pre-treatment in the 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;

105 Luengo et al., 2013; Parniakov et al., 2016; Pataro et al., 2017), while there are limited
106 data about the effect of PEF on the extraction of non-polar compounds (Luengo et al.,
107 2014; Roohinejad et al., 2014; Wiktor et al., 2015;Yin et al., 2008).
108 In particular, to date, only the study of Luengo et al. (2014) has addressed the PEF-
109 assisted extraction of lipid-soluble compounds, such as carotenoids, from tomato peels,
110 which have been treated by PEF after hand peeling of fresh tomatoes.
111 In addition, no studies have been published on the extractability of carotenoids from
112 tomato processed by-products (peels), after steam blanching (SB) of whole tomato fruits.
113 The objective of this work was to investigate the use of PEF, alone and in combination
114 with SB, as pre-treatment of whole tomato fruits, with the aim of improving the
115 extractability of carotenoids from tomato processing by-products (peels).
116 Specifically, the effects of different electric field strengths and steam blanching
117 emperatures on the cell disintegration index of peel tissue, as well as on the total content
118 and composition of carotenoids and antioxidant activity of the extracts were investigated.

119

120 **2. Materials & Methods**

121 2.1. Chemicals and raw material

122 Fully ripened tomatoes of the "*Pachino*" variety were purchased from a local supermarket
123 and stored in dark under refrigerated conditions (4±1 °C) until use, within 5 days from
124 purchase.

125 Color measurements were performed on the surface of tomatoes with a tristimulus
126 colorimeter CR400 Chroma Meter (Konika Minolta Inc., Japan). Five readings were
127 taken at random positions from each fruit. Data were collected in CIE L*a*b* color space

and the values of L*, a* and b* were recorded and used to evaluate the combination parameter hue (H) angle, which indicates the actual color or the redness.

Tomatoes of similar size (about 2.6 cm in diameter) and color (H=45.8±1.8) were selected prior to PEF, SB or PEF+SB pre-treatments, in order to use fruits that exhibited a homogeneous carotenoid concentration (Luengo et al 2014; Pataro et al., 2015).

Physical-chemical parameters of selected tomatoes, such as total soluble solids (6.53 ± 0.15 °Brix), titratable acidity (0.44 ± 0.05 g citric acid/100g fresh weight tomatoes) and moisture content (90.2 ± 0.8 g_{water}/100g fresh weight tomatoes), were also determined.

HPLC grade methanol and acetonitrile as well as acetone, iron (III) chloride hexahydrate ($FeCl_3 \cdot 6H_2O$), citric acid 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).

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

For each experiments, approximately 150 g of whole tomatoes (10 fruits) were subjected to PEF, SB or PEF+SB pre-treatments. In a first set of experiments, tomato fruits were loaded into the PEF treatment chamber with tap water at a constant solid to liquid ratio (1:1 g/mL) and exposed to 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 determined on the basis of preliminary experiments to ensure the preservation of the fruit integrity, and improve its peelability (TecnAlimentaria–Food Industry, 2017) while inducing a sufficient degree of cell membrane 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. All the PEF treatments were performed in triplicate.

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 the 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 the 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). All SB and PEF+SB treatments were performed in triplicates. After treatment, 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.

193 2.4. Cell disintegration index

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

206

207 $$Z_P = \frac{|Z_{untr(1kHz)}| - |Z_{tr(1kHz)}|}{|Z_{untr(1kHz)}| - |Z_{tr(1MHz)}|} \quad (1)$$

208

209 2.5. Determination of total carotenoid (TC) content

210 The total carotenoid (TC) content of tomato peels extracts from untreated and treated
211 samples was determined according to the method described by Lichtenthaler & Wellburn
212 (1983). The absorbance of undiluted extracts was measured at 470 nm (A_{470}), 645 nm
213 (A_{645}), and 662 nm (A_{662}), in a V-650 UV-Vis spectrophotometer (Jasco Inc., Easton,
214 USA). Absolute acetone was used as a blank. The total content of carotenoids, expressed

215 in mg/100 g of fresh weight (FW) peels, was calculated from the following equations for
216 100% acetone:

217 $$C_a = 11.75\ A_{662} - 2.35\ A_{645} \quad (2)$$

218 $$C_b = 18.61\ A_{645} - 3.96\ A_{662} \quad (3)$$

219 $$C_{x+c} = (1000\ A_{470} - 2.27\ C_a - 81.4\ C_b)/227 \quad (4)$$

220 where C_a is the content of chlorophyll a, C_b is the content of chlorophyll b, and C_{x+c} is the
221 content of carotenoids. All the assays were performed in triplicate.

222

223 2.6. Evaluation of ferric reducing antioxidant power (FRAP) of extracts

224 FRAP assay of tomato peels extracts was carried out according to the method described
225 by Benzie & Strain (1996) with some modification. Before the measurements, 0.3 M
226 sodium acetate buffer (pH 3.6) was prepared by dissolving 3.1 g of sodium acetate and
227 16 mL of acetic acid in 1000 mL of distilled water; 10 mM TPTZ solution was prepared
228 by dissolving 0.031 g TPTZ in 10 mL of 40 mM HCl; 20 mM ferric solution was
229 prepared by dissolving 0.054 g of $FeCl_3 \cdot 6H_2O$ in 10 mL of distilled water.
230 The FRAP working solution was prepared by freshly mixing 0.3 M sodium acetate
231 buffer, 10 mM TPTZ solution, and 20 mM ferric solution at a ratio of 10:1:1 (v/v/v). For
232 the analysis, 2.5 mL of freshly prepared FRAP working solution and 0.5 mL of undiluted
233 extract were mixed and incubated for 10 min at ambient temperature. The change in
234 absorbance due to the reduction of ferric-tripyridyltriazine (Fe III-TPTZ) complex by
235 the antioxidants contained in the samples was monitored at 593 nm using a V-650 UV-
236 Vis spectrophotometer (Jasco Inc., Easton, USA). The absorption 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. All the assays were performed in triplicate.

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 peel 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 peel 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, and 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's 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 carotenoid content and antioxidant power of tomato peel extracts

Figure 1 shows total carotenoid (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 previous observations showing 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, despite the compactness of the plant tissue (Zuorro et al., 2011), acetone is a good extraction solvent, because it is able 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 in the extracts, 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 degree of the cell membranes of the tomato peel tissues upon the exposure of the whole fruits to an external electric field was determined in terms of 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 from 0.25 (Z_p=0.20) to 0.50 kV/cm (Z_p=0.61), while a slight, not statistically significant increase was observed when the PEF intensity was increased to 0.75 kV/cm (Z_p=0.66). Remarkably, a highly positive correlation was observed between TC content and Z_P values (Table 1), which can be explained by 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 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), or betanin from red beets (Chalermchat et al., 2004; López et al., 2009). Moreover, Luengo et al. (2014), who investigated the extraction of carotenoid compounds from peels of fresh tomato (commercial variety: *tomate canario*), found that 90-μs PEF treatment at 5 kV/cm increased the extraction yields in acetone by 50%, as compared to a conventional solvent extraction. However, differently from this work, the authors applied PEF pre-treatment directly to the fresh

tomato peels rather than to tomato fruits, and found a lower concentration of carotenoids in the extracts (about 3.2 mg/100 g FW tomato peels). This could be attributed to the biological diversity of the tomatoes, which likely led to lower Z_P values (about 0.2 at 5 kV/cm and 90 μs), despite an applied field strength higher than this work.

A qualitative analysis of the composition of the peel extracts 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 appeared to be similar, suggesting that the electrical pre-treatment neither promoted the selective extraction of specific compounds nor caused isomerization or degradation reactions. 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 the HPLC chromatogram profiles of the extracts, probably due to the relatively mild intensity of the applied treatment (Kahmič-Kalamiza et al. 2014).

In particular, in Figure 2, the main peak can be 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 carotenoid content 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 the extracts from tomato peels.

Moreover, it is worth noting that, in comparison with the control sample, the application of PEF pre-treatment caused a remarkable increment of the lycopene 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 in the extracts from PEF treated blueberries and purple-fleshed potato (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 (66–372 %). In general, the higher the field strength, the greater the antioxidant power, but 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 (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 contributes to the antioxidant activity of the extracts.

The results of this study hence suggest that, within the range of field strength investigated, the cell disintegration level (Z_p = 0.61) achieved with the intermediate PEF treatment intensity (0.5 kV/cm) corresponds to the most favorable conditions to intensify the extractability of carotenoid compounds with the highest antioxidant activity. It is likely that higher PEF treatment intensity (>0.75 kV/cm) might further enhance the Zp value of peel tissues and, consequently, improve the extraction yield of valuable intracellular compounds. However, the application of more severe PEF treatment conditions seriously impair the integrity of tomato fruits (data not shown), which is in contrasts with the aim

354 of achieving, in addition to the valorization of tomato by-products, also high quality
355 peeled tomatoes, as envisaged in the FieldFood project.
356 Further investigations of PEF pre-treatment in combination with SB of tomato fruits
357 were, therefore, carried out at 0.5 kV/cm with a constant energy input of 1 kJ/kg.

358

359 *3.2. Combined effect of PEF and SB pre-treatments on Z_p, carotenoid content and*
360 *antioxidant power of tomato peels extracts*

361 Steam blanching (SB) is a unit operation typically used to facilitate peel removal from
362 tomato fruits during the manufacturing of several tomato products. Therefore, in view of
363 the exploitation as a cheap and rich source of natural carotenoids of the large amounts of
364 tomato processed by-products (peels) currently produced at the industrial level, the
365 impact of SB pre-treatment on the cell structure of peel tissues and the subsequent
366 recovery of these compounds should be evaluated. Eventually, the application of a mild
367 cell disintegration technique such as PEF in combination with SB of tomato fruits could
368 be used to further intensify the extractability of valuable intracellular compounds.
369 In this work, extracts obtained from peels of whole tomato fruits pre-treated by SB (1
370 min) alone or by the sequence of PEF (E=0.50 kV/cm, W_T=1 kJ/kg) and SB (1 min) at
371 different steam blanching temperature (50, 60 and 70 °C), were analyzed in order to
372 evaluate the impact of either the single thermal treatment or the combined treatment on
373 the extractability of carotenoid compounds with high antioxidant activity.
374 The results of Figure 4 show that the extraction yield of carotenoids from peels of mildly
375 SB-treated fruits was significantly improved (60-189%), as compared with the control
376 extraction performed from fresh tomato peels (Figure 1). However, no significant

377 difference was detected between the TC content of the SB-treated samples at 50 and 60
378 °C, whereas a significant ($p \leq 0.05$) difference was observed when the blanching
379 temperature was increased from 60 to 70 °C.
380 It is likely that in the blanching temperature range examined, the improved extractability
381 of carotenoids when increasing temperature can be related to the thermal damage induced
382 at the cuticular level (Strati & Oreopoulou, 2011a). In fact, as shown in Figure 5, the Z_P
383 values of tomato peels obtained from the SB pre-treatment of tomato fruits at 50, 60, and
384 70 °C, increased to 0.2, 0.36, and 0,57, respectively, with a significant difference
385 observed only when the temperature was increased from 50 to 70 °C. Moreover, a strong
386 positive correlation was observed between Z_p and TC content (Table 2). To the best of
387 our knowledge, no previous work investigated the effect of SB of tomato fruits on the
388 extractability of carotenoids from the peel residues, while several works dealt with the
389 effect of the extraction temperature on the recovery of carotenoids. To this purpose, for
390 example, Strati and Oreopoulou (2011a) observed that an increase of extraction
391 temperature from 25 to 70 °C caused an increase in the carotenoids concentration in
392 acetone extracts from tomato peel powder, which was attributed to the destruction of the
393 cellular structure.
394 In contrast, when PEF pre-treatment was applied prior to SB, the TC content rose to
395 significantly higher values ($p \leq 0.05$) with respect to the thermally treated samples for
396 blanching temperatures of 50 and 60 °C, while a slight but not significant increase was
397 observed when the temperature was increased to 70 °C. No statistical difference was,
398 instead, observed among the PEF+SB treated samples (Figure 4).
399 However, it is worth noting that the combined treatment showed an additive effect in the
400 extraction yield of TC at the blanching temperature of 50 °C, whereas a synergistic effect

401 was observed at 60 °C, corresponding to a maximum value of 37.9 mg/100 g FW tomato
402 peels. Further increasing the SB temperature up to 70 °C, instead, caused a slight but not
403 significant decrease in the amount of TC extracted, as compared with the combined
404 treatment performed at lower temperatures. From these results, it might be concluded that
405 the electroporation effect induced by PEF prior to the thermal treatment enables the
406 intensified recovery of valuable compounds at lower blanching temperature, probably
407 because of the reduced thermal stress that could negatively affect the extraction and
408 bioavailability of thermolabile compounds. Similarly, previously published works
409 demonstrated that PEF permeabilization of plant tissue before extraction has the potential
410 of decreasing the extraction temperature without affecting the extraction yield (Loginova
411 et al., 2011; López et al., 2009; Puértolas et al., 2013).
412 The results of Figure 4 positively correlate with the higher values of Z_P detected when
413 PEF was applied prior to SB treatment (Figure 5, Table 2), indicating that the combined
414 treatment has the potential to further enhance the degree of structural damages at the
415 cuticular level, thus facilitating the penetration capacity of the solvent and the recovery
416 of the carotenoid compounds.
417 Moreover, the results of Figure 4 are consistent with the HPLC chromatogram profiles of
418 the extracts obtained upon the application of SB (Figure 6a) alone or of PEF+SB (Figure
419 6b). Interestingly, it can be observed that, once again, only the peak of lycopene was
420 detected and that no isomerization or degradation occurred upon the application of either
421 a mild SB treatment or the combination of PEF with SB. In contrast, SB or PEF+SB
422 increased the yield compared to the extraction from untreated fresh peels or peels obtained
423 upon the PEF pre-treatment of tomato fruits (Figure 2). In particular, the results of Figure
424 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 increase in the antioxidant activities 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

449 a stronger correlation especially for samples obtained from fruits treated by SB alone
450 (Table 2).

451

452 **4. Conclusions**

453 The results of this study have demonstrated the efficacy of the pre-treatment of whole
454 tomato fruits, typically applied to facilitate tomato peelability, also on the extractability
455 of carotenoids from tomato peels. In particular, the cell disintegration induced at the
456 cuticular level by either the electrical and/or thermal treatment improves the penetration
457 of the solvent into the cytoplasm and the subsequent mass transfer of the solubilized
458 intracellular pigments, thus intensifying the extractability of carotenoid compounds.
459 More specifically, the application of a pulsed electric field treatment (E = 0.5 kV/cm; W_T
460 = 1 kJ/kg; T) prior to steam blanching of tomato fruits at 60 °C, exhibited a synergistic
461 effect on promoting the extraction yield of TC. HPLC analyses revealed that lycopene
462 was the most predominant carotenoid in the peel extracts, hence responsible for their
463 antioxidant activity. Moreover, these analyses also showed no evidence of isomerization
464 or degradation of lycopene upon the application of the electrical and/or thermal pre-
465 treatment.
466 This work demonstrates the potential of PEF pre-treatment, in combination with mild
467 steam blanching, to be implemented in the industrial processing of tomato fruits, to
468 achieve the valorization of the tomato processing by-products.
469 However, further studies are required to fully validate the implementation of PEF
470 technology at industrial scale, through the extension of this research to other tomato

471 cultivars in semi-industrial/real industrial conditions, also as a function of the ripening
472 stage of the raw material.

473

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476

REFERENCES

Barba, F.J., Parniakov, O., Pereira, S.A., Wiktor, A., Grimi, N., Boussetta, N., Saraiva, J.A., Raso, J., Martin-Belloso, O., Witrowa-Rajchert, D., Lebovka, N., Vorobiev, E. (2015). Current applications and new opportunities for the use of pulsed electric fields in food science and industry. *Food Research International*, 77, 773-798.

Benzie, I. F., & Strain, J. J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. *Analytical Biochemistry*, 239, 70 – 76.

Bobinaitė, R., Pataro, G., Lamanauskas, N., Šatkauskas, S., Viškelis, P., & Ferrari, G. (2015). Application of pulsed electric field in the production of juice and extraction of bioactive compounds from blueberry fruits and their by-products. *Journal of Food Science & Technology*, http://dx.doi.org/10.1007/s13197-014-1668-0.

Boussetta, N., Vorobiev, E., Le, L.H., Cordin-Falcimaigne, A., & Lanoiselle, J.-L. (2012). Application of electrical treatments in alcoholic solvent for polyphenols extraction from grape seeds. *LWT - Food Science and Technology*, 46, 127 – 134.

Chalermchat, Y., Fincan, M., & Dejmek, P. (2004). Pulsed electric field treatment for solid-liquid extraction of red beetroot pigment: mathematical modelling of mass transfer. *Journal of Food Engineering*, 64, 229-236.

Chemat, F., Rombaut, N., Meullemiestre, A., Turk, M., Perino, S., Fabiano-Tixier, A. S., & Abert-Vian, M. (2017). Review of Green Food Processing techniques. Preservation, transformation, and extraction. *Innovative Food Science and Emerging Technologies*, 41, 357-377.

Corrales, M., Toepfl, S., Butz, P., Knorr, D., & Tauscher, B. (2008). Extraction of anthocyanins from grape by-products assisted by ultrasonic, high hydrostatic pressure or

500 pulsed electric field: A comparison. *Innovative Food Science and Emerging*
501 *Technologies*, 9, 85 – 91.
502 Donsì, F., Ferrari, G., & Pataro, G. (2010). Applications of Pulsed Electric Field
503 Treatments for the Enhancement of Mass Transfer from Vegetable Tissue. *Food*
504 *Engineering Reviews*, 2, 109 – 130.
505 Donsi, F., Ferrari, G., Fruilo, M., & Pataro, G. (2011). Pulsed Electric Field-Assisted
506 Vinification of Aglianico and Piedirosso Grapes. *Journal of Agricultural and Food*
507 *Chemistry* , 58, 11606–11615.
508 FAOSTAT (Food and Agriculture Organization of the United Nations), (2013).
509 http://www.fao.org/faostat/en/#data/QC.
510 Gachovska, T., Cassada, D., Subbiah, J., Hanna, M., Thippareddi, H., & Snow, D. (2010).
511 Enhanced Anthocyanin Extraction from Red Cabbage Using Pulsed Electric Field
512 Processing. *Journal of Food Science*, 75, E323-E329.
513 Kahmič-Kalamiza, S., Vorobiev, E., & Miklavčič, D. (2014). Electroporation in food
514 processing and biorefinery. *The Journal of Membrane Biology*, 247, 1279–1304.
515 Knoblich, M., Anderson, B., & Latshaw, D. (2005). Analyses of tomato peel and seed
516 byproducts and their use as a source of carotenoids. *Journal of the Science of Food and*
517 *Agriculture,* 85, 1166–1170.
518 Koubaa, M., Barba, F.J., Grimi, N., Mhemdi, H., Koubaa, W., Boussetta, N., & Vorobiev,
519 E. (2016). Recovery of colorants from red prickly pear peels and pulps enhanced by
520 pulsed electric field and ultrasound. *Innovative Food Science & Emerging Technologies*,
521 37, 336-344.
522 Lenucci, M.S., De Caroli, M., Marrese, P.P., Iurlaro, A., Rescio, L., Bohm, V.,
523 Dalessandro, G., & Piro, G. (2015). Enzyme-aided extraction of lycopene from high-

524 pigment tomato cultivars by supercritical carbon dioxide. *Food Chemistry*, 170, 193 –
525 202.
526 Lichtenthaler, H., Wellburn, A. (1983). Determination of Total Carotenoids and
527 Chlorophyll A and B of Leaf Extracts in Different Solvents. *Biochemical Society*
528 *Transactions*, 11, 591 – 592.
529 Lin, C. H., & Chen, B. H. (2003). Determination of carotenoids in tomato juice by liquid
530 chromatography. *Journal of Chromatography*, 1012, 103 – 109.
531 Loginova, K. V., Vorobiev, E., Bals, O., & Lebovka, N. I. (2011). Pilot study of
532 countercurrent cold and mild heat extraction of sugar from sugar beets, assisted by
533 pulsed electric fields. *Journal of Food Engineering*, 102, 340–347.
534 Lopéz, N., Puertolas, E., Condon, S., Raso, J., & Alvarez, I. (2009). Enhancement of
535 the extraction of betanine from red beetroot by pulsed electric fields. *Journal of Food*
536 *Engineering*, 90, 60-66.
537 Luengo, E., Alvarez, A., & Raso, J. (2013). Improving the pressing extraction of
538 polyphenols of orange peel by pulsed electric fields. I*nnovative Food Science &*
539 *Emerging Technology*, 17, 79 – 84.
540 Luengo, E., Alvarez, I., & Raso, J. (2014). Improving carotenoids extraction from
541 tomato waste by pulsed electric fields. *Frontiers in Nutrition*, 1, 1 – 10.
542 MarketsandMarkets, (2016). Carotenoids Market by Type (Astaxanthin, Beta-
543 Carotene, Canthaxanthin, Lutein, Lycopene, & Zeaxanthin), Source (Synthetic and
544 Natural), Application (Supplements, Food, Feed, and Cosmetics), & by Region -
545 Global Trends & Forecasts to 2021. https://www.marketsandmarkets.com/Market-
546 Reports/carotenoid-market-158421566.html.

547 Naviglio, D., Pizzolongo, F., Santini, A., Ferrara, L., & Naviglio, B. (2006).
548 Estrazione del licopene ad elevato grado di purezza dagli scarti di pomodoro.
549 *Ingredienti Alimentari*, 5, 11 - 14.
550 Pan, Z., Li, X., Bingol, G., McHugh, T., & Atungulu, G. (2009). Development of infrared
551 radiation heating method for sustainable tomato peeling. *Applied Engineering in*
552 *Agriculture*, 25, 935–941.
553 Parniakov, O., Barba, F.J., Grimi, N., Lebovka, N., & Vorobiev, E. (2016). Extraction
554 assisted by pulsed electric energy as a potential tool for green and sustainable recovery
555 of nutritionally valuable compounds from mango peels. *Food Chemistry*, 192, 842 – 848.
556 Pataro, G., Sinik, M., Capitoli, M. M., Donsì, F, & Ferrari, G. (2015). The influence of
557 Post-harvest UV-C and Pulsed Light treatments on quality and antioxidant properties of
558 tomato fruits during storage. *Innovative Food Science and Emerging Technologies*, 30,
559 103–111.
560 Pataro G., Bobinaité, R., Bobinas, Č., Šatkauskas, S., Raudonis, R., Visockis, M., Ferrari,
561 G., & Viskelis, P. (2017). Improving the Extraction of Juice and Anthocyanins from
562 Blueberry Fruits and Their By-products by Application of Pulsed Electric Fields. *Food*
563 *Bioprocess Technology*, http://dx.doi.org/10.1007/s11947-017-1928-x.
564 Puertolas, E., Cregenzan, O., Luengo, E., Alvarez, I., & Raso, J. (2013). Pulsed –
565 electric – field – assisted extraction of anthocyanins from purple - fleshed potatoes.
566 *Food Chemistry*, 136, 1330 – 1336.
567 Queralt, A.V., Oms Oliu, G., Odriozola-Serrano, I., Lamuela-Raventos, R.M., Martin-
568 Belloso, O., & Elez-Martinez, P. (2013). Metabolite profiling of phenolic and carotenoid
569 contents in tomatoes after moderate-intensity pulsed electric field treatments. *Food*
570 *Chemistry*, 136, 199 – 205.

571 Riggi, E. (2010). Recent patents on the extraction of carotenoids. *Recent Patents on Food,*
572 *Nutrition and Agriculture*, 2, 75-82.
573 Rock, C., Yang, W., Goodrich-Schneider, R., & Feng, H. (2012). Conventional and
574 alternative methods for tomato peeling. *Food Engineering Reviews*, 4, 1 - 15.
575 Roohinejad, S., Oey, Y., & Everett, D. W. (2014). Effect of pulsed electric field
576 processing on carotenoid extractability of carrot purée. *International Journal of Food*
577 *Science & Technology*, 49, 2120-2127.
578 Rossini, G., Toscano, G., Duca, D., Corinaldesi, F., Foppa Pedretti, E., & Riva, G. (2013).
579 Analysis of the characteristics of the tomato manufacturing residues finalized to the
580 energy recovery. *Biomass and Bioenergy*, 51, 177 – 182.
581 Singh, A., Ahmad, S., & Ahmad, A. (2015). Green extraction methods and environmental
582 applications of carotenoids-a review. *The Royal Society of Chemistry Advances*, 5, 62358
583 – 62393.
584 Strati, I.F., & Oreopoulou, V. (2011a). Effect of extraction parameters on the carotenoid
585 recovery from tomato waste. *International Journal of Food Science and Technology*, 46,
586 23 – 29.
587 Strati, I.F., & Oreopoulou, V. (2011b). Process optimisation for recovery of carotenoids
588 from tomato waste. *Food Chemistry*, 129, 747 – 752.
589 Strati, I.F., & Oreopoulou, V. (2014). Recovery of carotenoids from tomato processing
590 by-products – a review. *Food Research International*, 65, 311 – 321.
591 TecnAlimentaria–Food Industry (2017). "The field food project", N° 8, December 2017,
592 210-211. http://www.tecnalimentaria.it/category/tecnalimentaria-food-industry/.
593 Wiktor, A., Sledz, M., Nowacka, M., Rybak, K., Chudoba, T., Lojkowski, W., & Witrova-
594 Rajchert, D. (2015). The impact of pulsed electric field treatment on selected bioactive

595 compound content and color of plant tissue. *Innovative Food Science & Emerging*
596 *Technologies*, 30, 69-78.
597 Yin, Y., Cui, J., & Ding, H. (2008). Optimization of betulin extraction process from
598 Inonotus Obliquus with pulsed electric fields. *Innovative Food Science & Emerging*
599 *Technologies*, 9, 306-310.
600 Zuorro, A., Fidaleo, M., Lavecchia, R. (2011). Enzyme-assisted extraction of lycopene
601 from tomato processing waste. *Enzyme and Microbial Technology*, 49, 567 – 573.

Fig.1

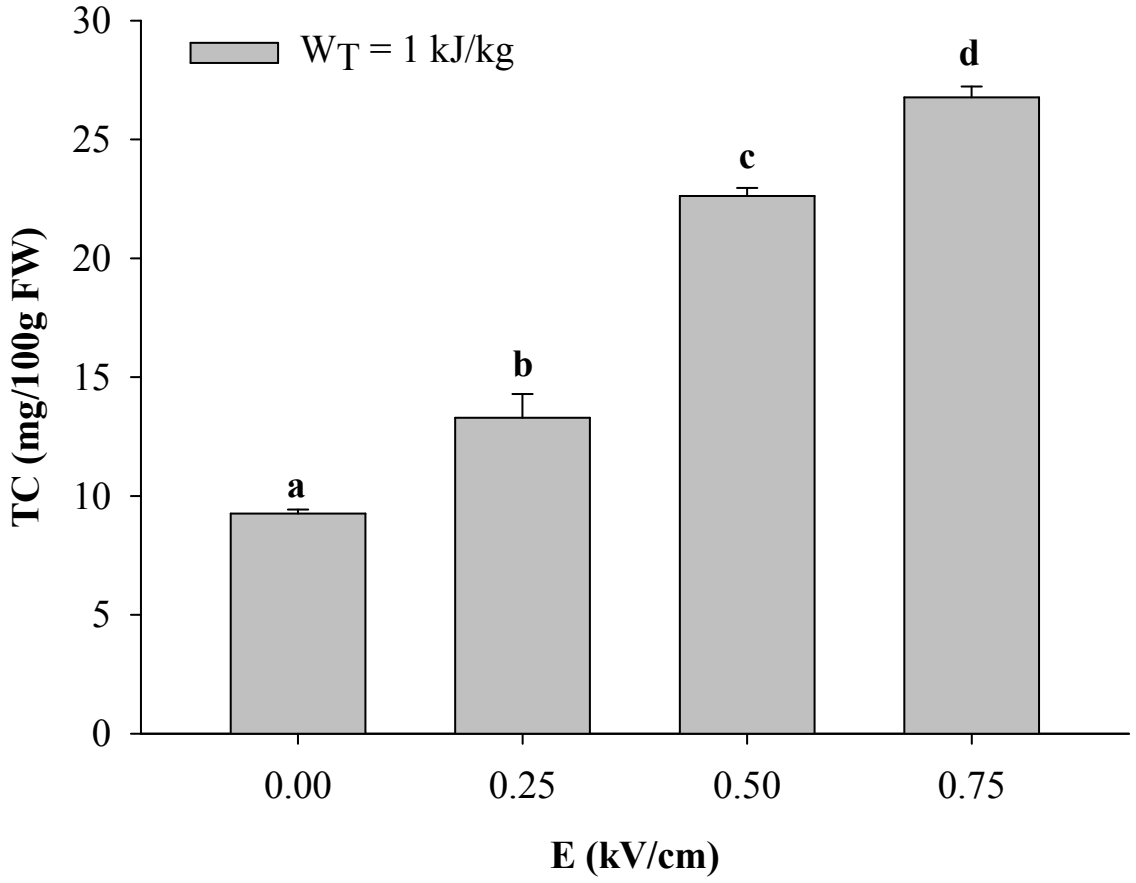
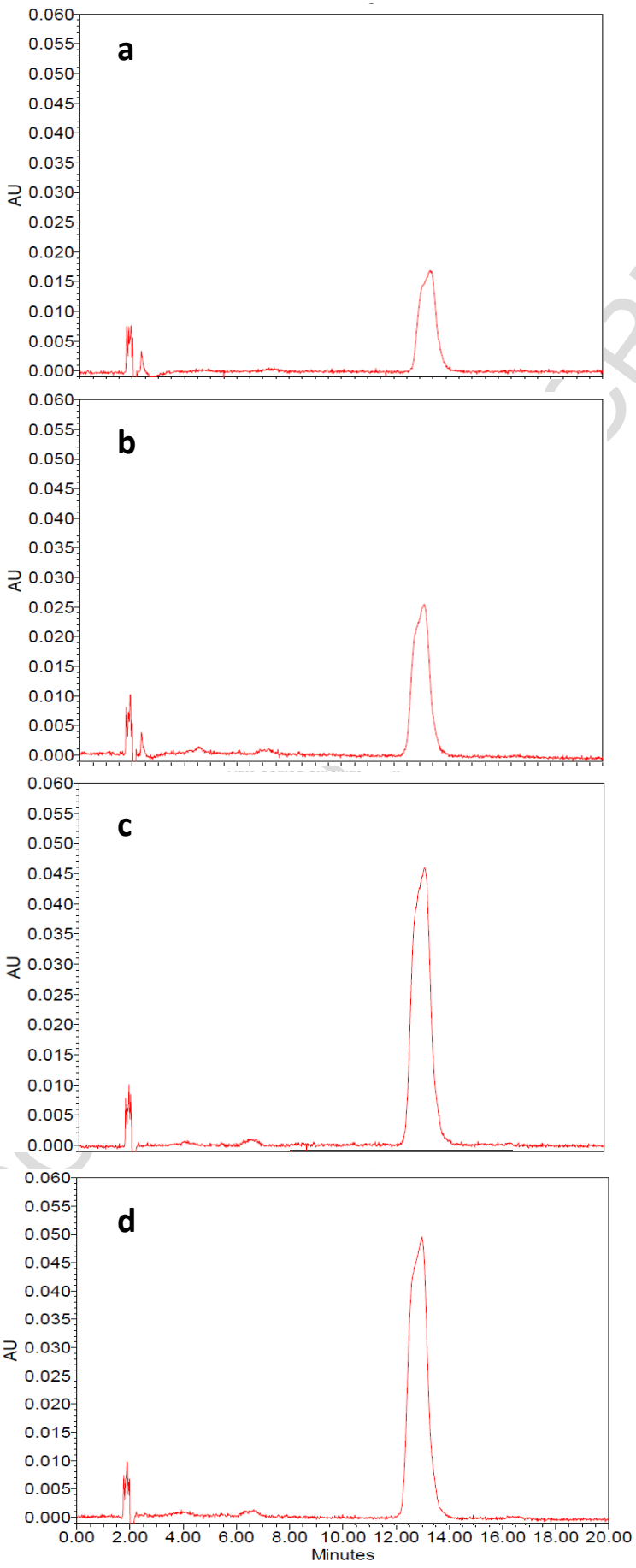


Fig. 2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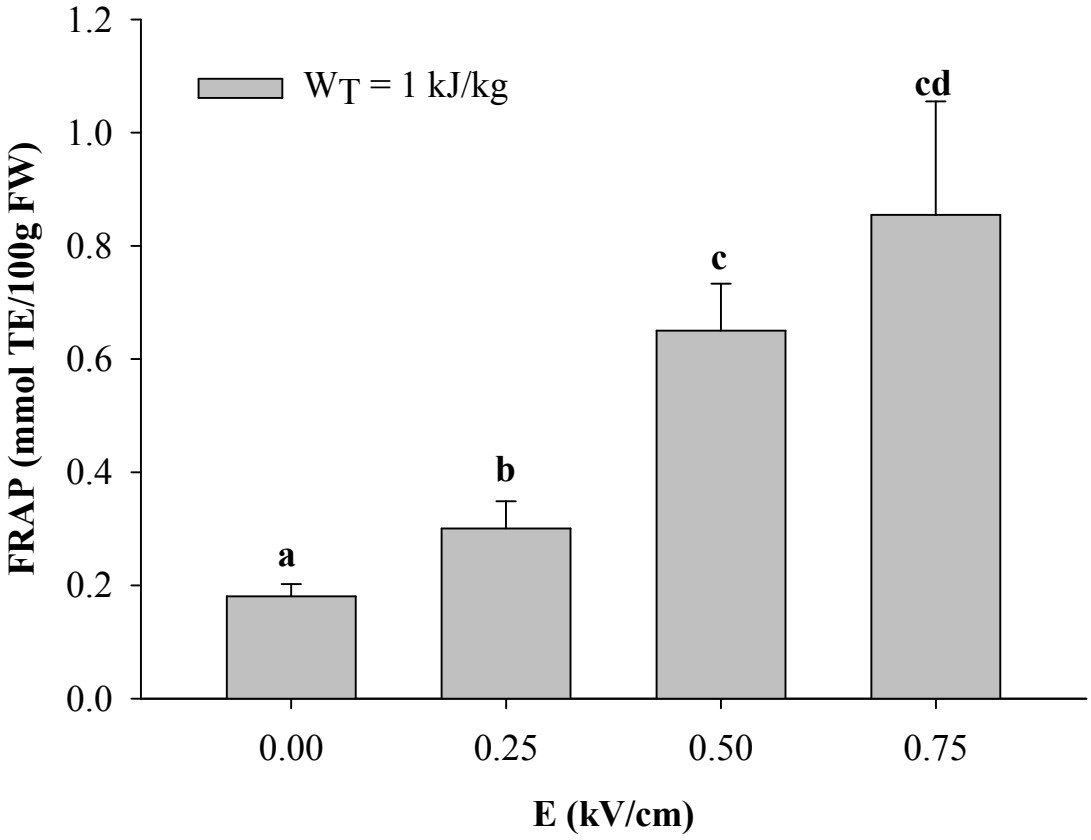
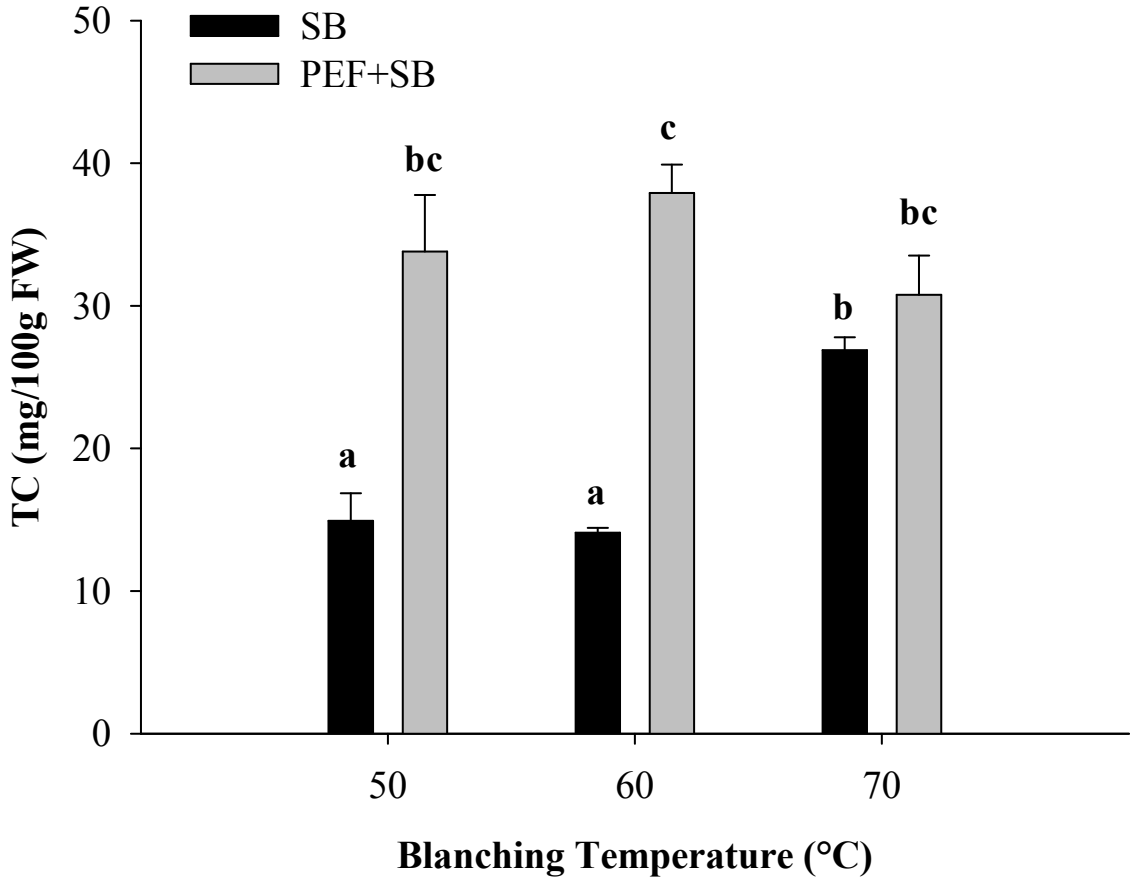


Fig. 4



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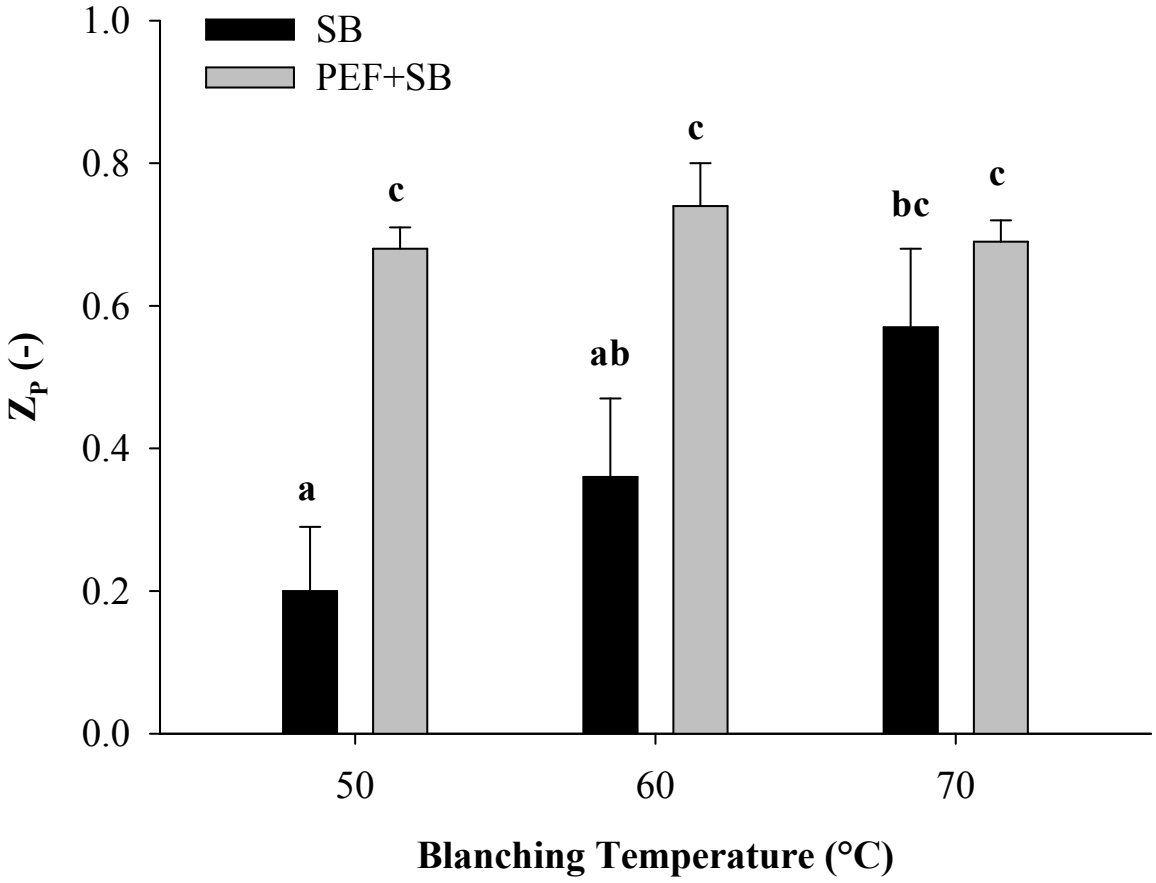

Fig. 6a

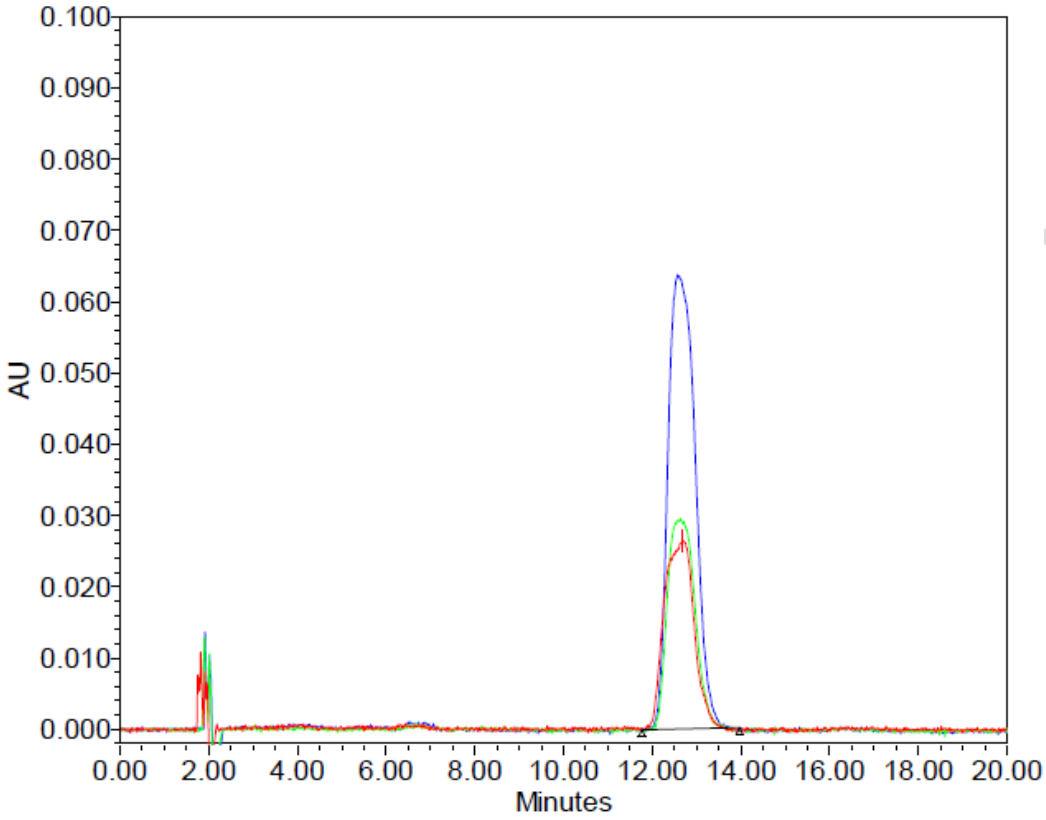
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Fig. 6b

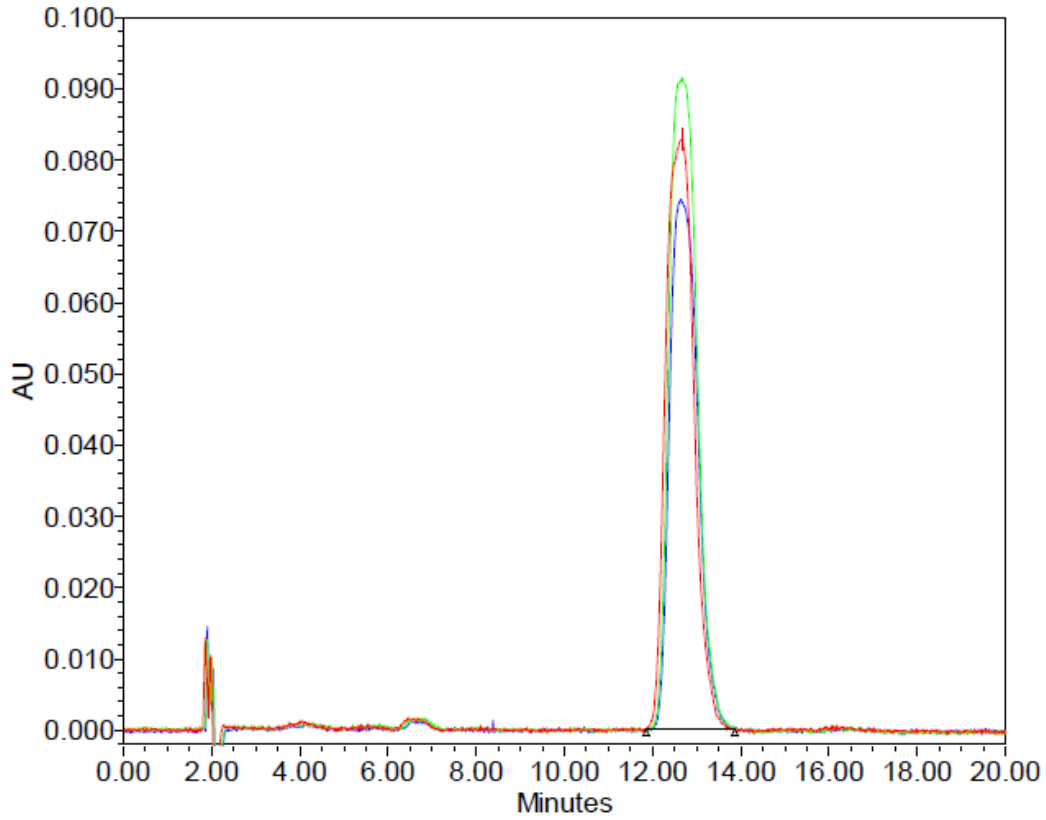
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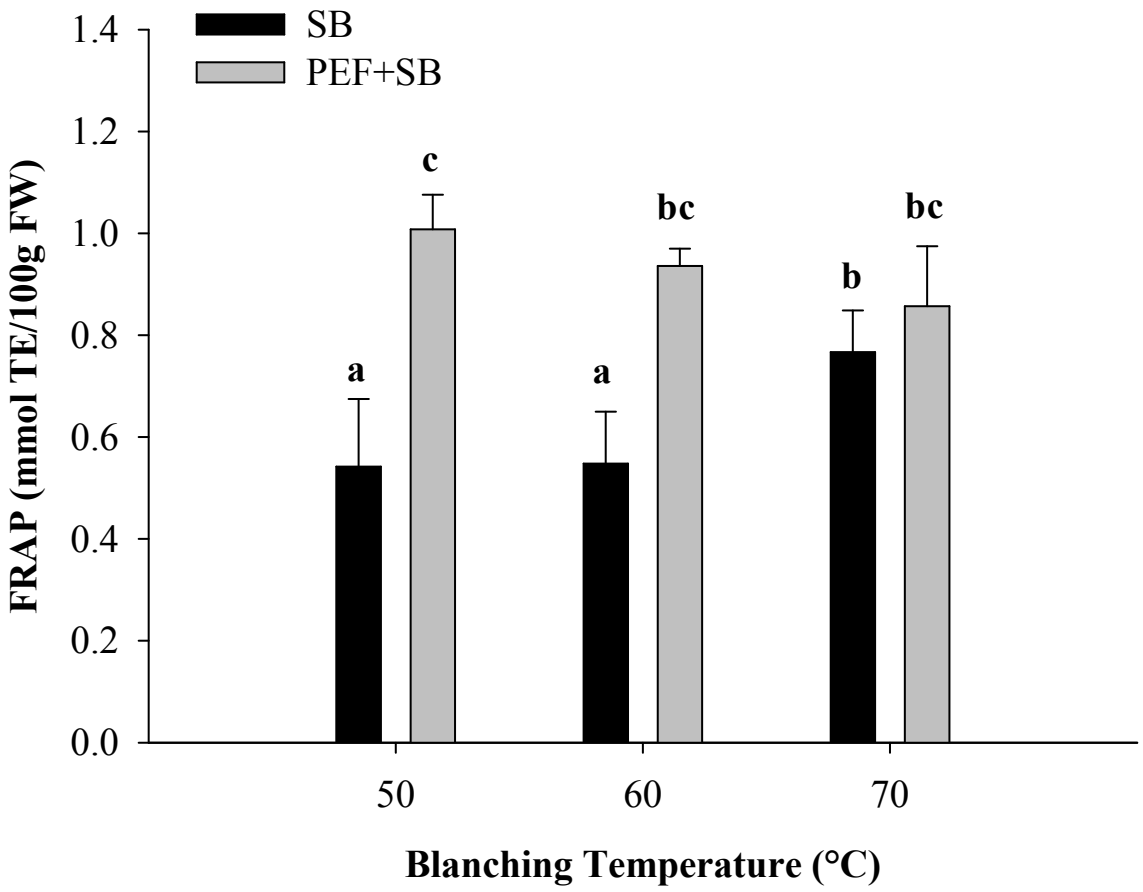
Fig. 7



Highlights

- PEF is combined with steam blanching (SB) to intensify carotenoids extraction
- PEF and SB treatments of whole tomatoes induced peel damages at cuticular level
- Combined PEF and SB showed a synergistic effect on carotenoids recovery from peels
- All-trans-lycopene was the most abundant carotenoid in tomato peels extract
- PEF and SB did not cause any degradation/isomerization of lycopene

Table 1 Correlation coefficient among cell disintegration index (Z_p) 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	1.000	0.978	0.961	0.994
TCC	0.978	1.000	0.997	0.998
AA	0.961	0.997	1.000	0.991
Lyc	0.994	0.998	0.991	1.000

Table 2. Correlation coefficient among cell disintegration index (Z_p) 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, W_T=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	1.000	0.876	0.830	0.912	-0.128	0.906	0.705
TCC (SB)	0.876	1.000	-	0.997	-	0.998	-
TCC (PEF-SB)	0.830	-	1.000	-	0.447	-	0.981
AA (SB)	0.912	0.997	-	1.000	-	1.000	-
AA (PEF-SB)	-0.128	-	0.447	-	1.000	-	0.613
Lyc (SB)	0.906	0.998	-	1.000	-	1.000	-
Lyc (PEF-SB)	0.705	-	0.981	-	0.613	-	1.000