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Effect of PEF pre-treatment and extraction temperature on the recovery of carotenoids from tomato wastes

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In this work, the influence of pulsed electric fields (PEF) pre-treatment and extraction temperature on the recovery of carotenoid compounds from tomato peels, achieved after peeling of steam blanched whole tomato fruits, was investigated. PEF pre-treatments were carried out at different field strengths (E = 0.5–5 kV/cm) and total specific energy input (W_T = 0.5-20 kJ/kg), with fixed pulse width (20 μs) and frequency (10 Hz). The cell disintegration index (Z_P) was used to identify the optimal PEF processing conditions for the pre-treatment of tomato peels before the extraction with acetone at different temperatures (20-50°C). Extracts from untreated and PEF treated samples were analysed in terms of content and composition of carotenoids and antioxidant power. According to the Z_P values, an energy input of 5 kJ/kg was enough for reaching the maximal permeabilization of tomato peel tissues at any investigated field strength. At this optimal energy input, higher field strengths yielded an increase in total carotenoids content and antioxidant power of the extracts obtained at 20°C. However, only the extracts obtained from PEF pre-treated peels at 5 kV/cm and 5 kJ/kg showed a significantly higher total carotenoids content (47.3%) and antioxidant power (68%), as compared to the untreated samples. Regardless of the application of a PEF pre-treatment, the increase of the extraction temperature did not lead to further intensification in the recovery yield of total carotenoids in the extracts. Moreover, HPLC analyses revealed that lycopene was the main carotenoid extracted and no degradation/isomerization phenomena occurred at the mild PEF treatment conditions and extraction temperature investigated. Results obtained in this work demonstrated that the PEF pre-treatment of tomato processing wastes coupled with room or moderate extraction temperature could add new value to the tomato processing chain, improving economic performances and decreasing waste problems.

1. Introduction

Tomato fruit is among the most widely consumed vegetables in the world and represents an important source of many bioactive compounds, especially carotenoids, able to reduce the risk of cancer and cardiovascular diseases (Lenucci et al., 2015). Millions of tomato tons are processed every year to obtain products such as peeled tomatoes for canning and dicing. Peeling is, therefore, one of the most critical phases in the industrial transformation of tomato fruits, which typically requires the use of steam blanching (SB) coupled with mechanical systems for peel removal (Arnal et al., 2018). Therefore, peels along with seeds and unused pulp, are the main wastes produced during industrial processing of tomato fruits, representing 2–5 % in weight of the total processed tomatoes (Knoblich et al., 2005). These wastes currently find low-added value uses as animal feed and fertilizers or are directly sent to landfill (Knoblich et al., 2005; Strati & Oreopoulou, 2014). However, they still retain a huge amount of several carotenoids with high antioxidant activity, especially lycopene, with potential industrial applications as natural pigment, food supplement or nutraceutical, as well as in the preparation of skin cosmetic products due its anti-aging properties (Lenucci et al., 2015; Strati & Oreopoulou, 2014). The recovery of carotenoids from plant tissues is usually based on a conventional extraction step using an organic solvent (e.g., hexane, acetone, ethanol, ethyl acetate) or a solvent mixture with high affinity for lipophilic compounds. However, in order to enhance the extraction yield, these methods often require energy-intensive pre-treatments of comminution and/or drying of the raw material, as well as relatively high extraction temperature and large volumes of organic solvents, which are very often potentially

toxic and harmful to the environment. In addition, they may induce either the loss of valuable compounds or the co-extraction of undesirable components, thus increasing the downstream processing costs (Luengo et al., 2014; Pataro et al., 2018). For these reasons, recent studies focused on applying process intensification concepts based on the implementation of wet cell disruption methods, such as pulsed electric fields (PEF), prior to extraction process, in order to lower the operational costs, reduce the environmental impact and achieve high yields of the desired products (Pataro et al., 2018). Specifically, many investigations have proved that the application of PEF pre-treatment of moderate electric field intensity (0.5-10 kV/cm) and relatively low energy input (1-10 kJ/kg) induces the permeabilization of cell membranes by electroporation, thus facilitating the access of the solvent to the intracellular compounds of interest and enhancing their recovery from a wide range of food processing wastes and by-products (Bobinaitė et al., 2015; Luengo et al., 2014; Pataro et al., 2018). Nevertheless, to our knowledge, only two works dealt with the use of PEF-assisted extraction process as an intensification pre-treatment in the extraction of carotenoids from tomato peels. However, none of them was addressed to study the extractability of carotenoids from tomato peels obtained after peeling of steam blanched tomato fruits. Specifically, Luengo et al. (2014) studied the PEF-assisted extraction of carotenoids from tomato peels treated by PEF after hand peeling of fresh tomatoes. In a more recent study, instead, Pataro et al. (2018) investigated the extractability of carotenoids from tomato peels, but only as a side benefit of PEF treatment of whole tomato fruits for more energy-efficient steam-assisted peeling.
Therefore, the objective of this work was to investigate the use of PEF pre-treatment to permeabilize cell membranes of tomato peels achieved after SB of whole tomato fruits prior to the solvent extraction step with the aim of intensifying the recovery yield of carotenoid compounds. Firstly, the effect of different combinations of field strength (E) and total specific energy input (W_T) on the cell permeabilization index of peel tissues was evaluated with the aim of defining optimal PEF pre-treatment conditions to be applied before the subsequent extraction phase. Then, the effect of the PEF-assisted extraction process carried out at different maceration temperatures on the total content and composition of carotenoids, as well as on the antioxidant activity of the extracts, was assessed.

2. Materials and methods

2.1 Chemicals, raw materials and sample preparation

Acetone, iron chloride and 2,4,6-tripyridyl-S-triazine (TPTZ) were purchased from Sigma-Aldrich (Sigma Aldrich, Steinheim, Germany). Sodium acetate and acetic acid were purchased, respectively, from Panreac (Panreac Quimica, Barcelona, Spain) and Fisher (Fisher Scientific, Rodano, Italy). Trolox for Ferric Reducing Antioxidant Power (FRAP) assay was purchased from Acros Organics (Geel, Belgium).
Tomatoes of "*Datterino*" variety were purchased from a local dealer and stored under refrigerated conditions (4±1 °C) until use, within 5 days from purchase. Before the experiments, tomatoes of similar size (about 3 cm in diameter) and colour (Hue angle= 45.2±1.6) were selected and subsequently steam blanched at 70°C for 1 min in a lab-scale steam oven (Minea, SO25P, France) to facilitate their hand peeling. Square shaped tomato peels (1 cm^2) were then used for carrying out both impedance analysis and PEF-assisted extraction tests.

2.2 PEF equipment

PEF treatments were performed by using the PEF unit previously described by Bobinaitė et al. (2015). Briefly, the system consists of a high voltage pulsed power (25 kV-500 A) generator (Modulator PG, ScandiNova, Uppsala, Sweden) able to deliver monopolar square wave pulses (3-25 μs, 1-450 Hz) through the plant tissue placed between the electrodes of a batch cylindrical treatment chamber (3 cm in diameter, electrode gap up to 5 cm). The actual voltage and current signals at the treatment chamber were measured by high voltage and current probes, connected to an oscilloscope. The maximum electric field intensity (E, kV/cm) and total specific energy input (W_T, kJ/kg) were calculated as reported in Bobinaitė et al. (2015).

2.3 Cell disintegration index

Cell disintegration index (Z_P) was determined via impedance analyses and used to quantify the degree of cell membrane permeabilization of tomato peel tissues induced by PEF treatments. Measurements of electrical complex impedance in frequency sweep (10^2 – 10^6 Hz) of untreated and PEF treated samples were carried out by loading 5 g of tomato peels into the PEF treatment chamber connected to an impedance analyser (Solartron 1260, UK). For each PEF treatment condition investigated (E=0.5–5 kV/cm, W_T=0.5–20 kJ/kg, 20 μs pulse duration, 10 Hz), 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 and treated tissue in the low (0.1 kHz) and high (1 MHz) frequency ranges, as previously described by Pataro et al. (2018).

2.4 PEF-assisted extraction

For each PEF-assisted extraction experiment, approximately 5 g of tomato peels were loaded into the treatment chamber and PEF pre-treated under the optimal conditions identified through the Z_p determinations. After the electrical treatment, 1 g of tomato peels sample was taken out from the treatment chamber and placed into a 200 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 under constant shaking at 160 rpm in a water bath set at three different temperatures (20°C, 35°C and 50°C). Temperatures higher than 50°C were not tested as the boiling point of the solvent is 56°C. Samples of untreated tomato peels of the same size and shape were subjected to the same extraction protocol without PEF and used as a control. The extracts obtained from untreated and PEF treated tomato peels were centrifuged (6500rpm, 10 min) to separate the supernatants, which were then filtered through 0.45 µm syringe filters. The final extracts were then stored at -20 °C until further analysis.

2.5 Determination of total carotenoids (TC) and ferric reducing antioxidant power (FRAP) of extracts

The total carotenoids (TC) content of acetone extracts from untreated and PEF-treated tomato peels was determined by using the method described by Lichtenthaler & Wellburn (1983). The absorbance of undiluted extracts was measured in a V-650 UV-Vis spectrophotometer (Jasco Inc., Easton, USA) at 470 nm (A_{470}), 645 nm (A_{645}), and 662 nm (A_{662}), which correspond to the absorption wavelengths of lycopene, chlorophyll *a* and chlorophyll *b*, respectively. Absolute acetone was used as a blank. The total carotenoids content, expressed as mg per 100g of fresh weight (FW) tomato peels, was calculated from the following equation:

$$TC = (1000 \cdot A_{470} - 1509.52 \cdot A_{645} + 295.67 \cdot A_{662})/227 \quad (1)$$

FRAP assay of tomato peels extracts was carried out according to the method thoroughly described by Pataro et al. (2018). Trolox was used as the standard for calibration curve and the FRAP values were expressed as mmol of Trolox equivalents (mmol TE) per 100g of FW tomato peels.

2.6 HPLC analysis of carotenoid compounds

For the identification of individual carotenoids, the acetone extracts from untreated and PEF treated tomato peels were analysed by high-performance liquid chromatography (HPLC) using the method described by Pataro et al. (2018). The identification of the major carotenoids was carried out by comparing their retention times and absorption spectra with those described in the literature data (Naviglio et al., 2006).

2.7 Statistical analysis

PEF treatments and analyses on collected samples were carried out in triplicate. The mean values and standard deviations (SD) of the experimental data were calculated. Differences among mean values were analyzed by means of a one-way ANOVA analysis using SPSS 20 software (SPSS Inc., Chicago, USA). When significant differences were detected, the Tukey test was performed to determine which particular means were significantly different among them ($p \leq 0.05$).

3. Results and discussion

3.1 Effect of PEF treatment on the cell disintegration index of tomato peel tissues

The Z_P has been successfully used as a reliable macroscopic indicator of the degree of cell membrane permeabilization in diverse fruits and vegetables tissues and to select the optimal PEF treatment conditions (Bobinaitė et al., 2015; Luengo et al., 2014). Figure 1 depicts the influence of the total specific energy input (W_T) and field strength (E) on the Z_P of peels obtained from steam blanched tomato fruits. Results show that, for each value of the field strength applied, the Z_P increased significantly with increasing the energy input up to approximately 5 kJ/kg. Further increments of W_T above this value scarcely affected Z_P, which tended to level off to a constant value. Moreover, at any energy input investigated, the extent of cell membrane permeabilization was affected by the field strength applied. In particular, at 5 kJ/kg the Z_P values exhibited a statistically significant increase ($p \leq 0.05$) when the field strength was increased within the investigated range (0.5-5 kV/cm), with the highest value of 0.54 being detected for the most intense treatment condition.

The characteristic trend of Z_P with increasing intensity of PEF treatment observed in this research is in good agreement with the findings of Luengo et al. (2014). These authors found that, regardless of the field strength applied, the Z_P of peels from fresh tomato (commercial variety: *tomate canario*) significantly increased with the PEF treatment time up to 90 µs. Furthermore, at this treatment time, the Z_P value at 3, 5 and 7 kV/cm increased to about 0.1, 0.15 and 0.25. The greater values of Z_P observed in our work could be attributed not only to the biological diversity of the tested tomatoes, but especially to the thermal damages induced at the cuticular level during the SB at 70°C of whole tomato fruits (Pataro et al., 2018). Therefore, it is likely that, in

our case, the thermal pre-treatment of whole tomatoes may have weakened the cellular structures of tomato skin tissues, thus making them more susceptible to the permeabilization effect of the subsequent PEF treatment.

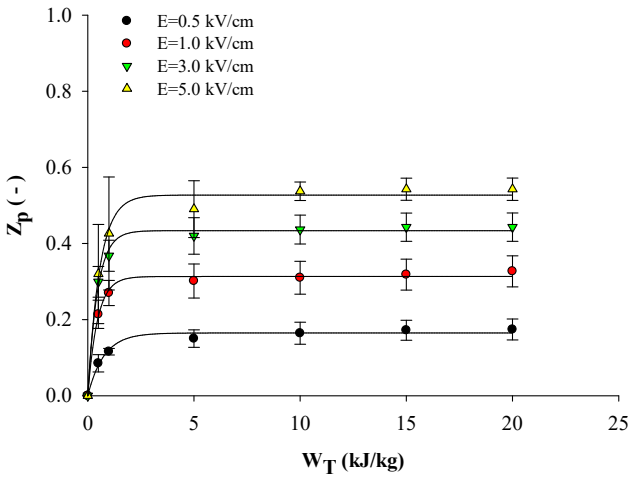

Figure 1: Cell disintegration index (Z_P) of tomato peels as a function of total specific energy input (W_T) and for different electric field strengths (E). Standard deviations were used as error bars.

According to these results, further investigations of PEF pre-treatment on the extractability of carotenoids from tomato peels were carried out at 0.5, 1, 3 and 5 kV/cm with a constant energy input of 5 kJ/kg.

3.2 Effect of PEF pre-treatment on the total carotenoids content and antioxidant power of tomato peel extracts

Table 1 shows the TC content and the FRAP values detected in the extracts of untreated (control, 0 kV/cm) and PEF treated tomato peels at different field strengths. The amount of TC extracted from the untreated samples was 54.6 mg/100g FW. This confirmed that a substantial amount of carotenoids were retained in the skins of steam blanched tomato fruits. Moreover, in agreement with previous findings (Pataro et al. 2018), the ability of acetone to penetrate the plant cells of tomato peels where carotenoids are enclosed, was likely enhanced by the partial cell disintegration induced at cuticular level by the mild SB treatment.

Table 1: Total carotenoids content (TC) and antioxidant power (FRAP) of acetone extracts obtained at 20°C from untreated (0 kV/cm) and PEF-treated (W_T = 5 kJ/kg) tomato peels at different field strengths (E). Different letters in the same row indicate significant differences among the mean values (p≤0.05).

E (kV/cm)	0	0.5	1	3	5
TC(mg/100g$_{FW}$)	54.6 ± 5.6^{a}	64.5 ± 8.1^{ab}	66.7 ± 8.9^{ab}	66.4 ± 0.5^{ab}	80.4 ± 2.2^{b}
FRAP(mmol$_{TE}$/100g$_{FW}$)	2.5 ± 0.1^{a}	3.1 ± 0.4^{ab}	3.2 ± 0.4^{ab}	3.2 ± 0.2^{ab}	4.2 ± 0.4^{b}

The application of PEF pre-treatment (0.5-5 kV/cm) to tomato peels markedly enhanced the TC content in extracts (by 18.1-47.3 %), as compared with the control extraction. However, significant differences (p≤0.05) were detected only when the field strength was increased at 5 kV/cm. It is likely that the electroporation effect was positively affecting the penetration of the solvent into the cell and the subsequent diffusion of the solubilized intracellular compounds (Luengo et al., 2014). This is corroborated by the strong positive correlation found between TC and Z_P values (r=0.90), indicating that the PEF treatment has the potential to furtherly enhance the degree of cell disintegration induced at the cuticular level by the previous SB treatment, thus intensifying the extractability of carotenoids. These results are consistent with those observed in the work of Luengo et al. (2014), who found that a 90 μs PEF treatment at 5 kV/cm yielded an increase in TC by about 50%, as compared with the control extraction. However, differently from this work, the authors used a different tomato variety and applied PEF pre-treatment to peels obtained from fresh tomatoes rather than steam blanched tomatoes, which likely led to a lower Z_P value (0.15) and, consequently, to a lower extraction yield of TC (3.2 mg/100g FW tomato peels). Furthermore, our results also showed that the extracts obtained from PEF-treated tomato peels possessed a stronger antioxidant power (24-68%) than the control extracts (Table 1), even though significant differences were detected only for samples treated at 5 kV/cm. This is not surprising being the antioxidant power of tomato peel extracts mainly related to the content of carotenoid compounds (Luengo et al. 2014; Pataro et al., 2018), as confirmed by the highly positive correlation found between TC and FRAP values (r=0.99).

According to the results of Table 1, in additional studies that aimed at investigating the effect of the extraction temperature on the recovery yields of carotenoids, the PEF conditions were set at 5 kV/cm and 5 kJ/kg.

3.3 Effect of extraction temperature on the total carotenoids content and antioxidant power of tomato peels extracts

The influence of the extraction temperature (20–50 °C) on the TC content and FRAP values of extracts from untreated and PEF-treated (E=5 kV/cm, W_T=5 kJ/kg) tomato peels is presented in Table 2. Results show that the increase in the extraction temperature did not significantly ($p>0.05$) increase the TC content in the control extracts, even though a slight increment (4-13 %) in the recovery yield of carotenoids was observed for temperature higher than 35°C. It is likely that the increase in the solubility of the material being extracted and its diffusivity with temperature could explain the slight improvement in the extractability of carotenoids.

Table 2: Total carotenoids content (TC) and antioxidant power (FRAP) of acetone extracts from untreated (Control) and PEF-treated (E=5 kV/cm; W_T=5 kJ/kg) tomato peels as a function of the extraction temperature. Different letters in the same row indicate significant differences among the mean values ($p\leq0.05$).

Sample	Control			PEF		
Temperature (°C)	20	35	50	20	35	50
TC(mg/100g_{FW})	54.6 ± 5.6^{a}	58.8 ± 7.2^{a}	61.8 ± 7.6^{a}	80.4 ± 2.2^{b}	79.3 ± 1.9^{b}	84.0 ± 8.3^{b}
FRAP(mmol$_{TE}$/100g_{FW})	2.5 ± 0.1^{a}	3.4 ± 0.5^{ab}	3.5 ± 0.2^{ab}	4.2 ± 0.4^{b}	4.0 ± 0.2^{b}	5.2 ± 0.4^{c}

Independently on the extraction temperature, PEF pre-treatment significantly ($p\leq0.05$) increased the TC content of extracts (35-47%), as compared to the control extraction. These results reflect a positive effect of PEF application for the intensification of the extractability of carotenoids. However, it is worth noting that, among the PEF treated samples, the increase of the extraction temperature from 20 to 50 °C did not contribute to a significant increase in the extraction yield of TC. To date, no previous works investigated the combined effect of PEF-pre-treatment and extraction temperature on the extractability of carotenoids from the peels of steam blanched tomato fruits, while several works dealt with the effect of the extraction temperature on the recovery of carotenoids. For instance, Strati and Oreopoulou (2011) observed that an increase of extraction temperature from 25 to 50 °C caused a significant increase in the carotenoids concentration in acetone extracts from tomato peel powder. This improved extractability of carotenoids with temperature was related to cellular structure damages induced by the mild thermal effect. These findings seem apparently in contrast with our results, where likely the structural damages induced at cuticular level of the peel tissue by either SB or SB followed by PEF treatment masked the damages induced by the moderate extraction temperature examined. From these results, it might be concluded that PEF permeabilization of peel tissues has the potential of decreasing the extraction temperature without affecting the recovery yield, which is consistent with findings previously reported by other scientists on different plant tissues (Puértolas et al., 2013). Figure 2 compares the HPLC chromatogram profiles for the extracts obtained from untreated and PEF treated tomato peels after extraction at 20 and 50°C. Similar HPLC profiles were obtained at the intermediate temperature of 35°C (data not shown). The profiles of the extracts from untreated and PEF-treated samples appeared to be similar, independently on the extraction temperature. Only the peak of all-trans lycopene was detected at an elution time of 12.65 min (Naviglio et al., 2006), which is consistent with the fact that lycopene is the most abundant carotenoid in tomato processing peels (Pataro et al., 2018, Strati & Oreopoulou, 2011). Moreover, in agreement with previous findings (Luengo et al., 2014; Pataro et al., 2018; Strati & Oreopoulou, 2011), HPLC analysis of our extracts indicated that, at the mild extraction temperatures and PEF treatment conditions examined, neither selective extraction of specific compounds nor isomerization/degradation of individual carotenoids occurred. The only differences observed were that either PEF pre-treatment or extraction temperature affected the lycopene peak area. In particular, coherently with the results of Table 2, in comparison with the control samples, the application of PEF pre-treatment caused a remarkable increment of the lycopene peak area of about 32%, independently of the extraction temperature investigated, while regardless of the application of PEF treatment, an increase in the extraction temperature from 20 to 50°C increased the lycopene peak area by about 22%. Additionally, as shown in Table 2, the extracts obtained from the PEF-treated tomato peels possessed a higher antioxidant power (18-68 %) than the control extracts, but significant differences ($p\leq0.05$) were detected only at the extraction temperature of 20 and 50 °C. The FRAP values correlate well with the higher content of carotenoids in the extracts ($r=0.84$), which clearly indicates that carotenoids, especially lycopene (Figure 2), predominantly contribute to the antioxidant power of extracts. However, it is worth noting that a significantly ($p\leq0.05$) higher antioxidant power was detected in extracts from the PEF-treated samples obtained at the highest extraction temperature, likely due to the co-extraction of polyphenolic and non-phenolic substances, which may have contributed to the overall antioxidant power.

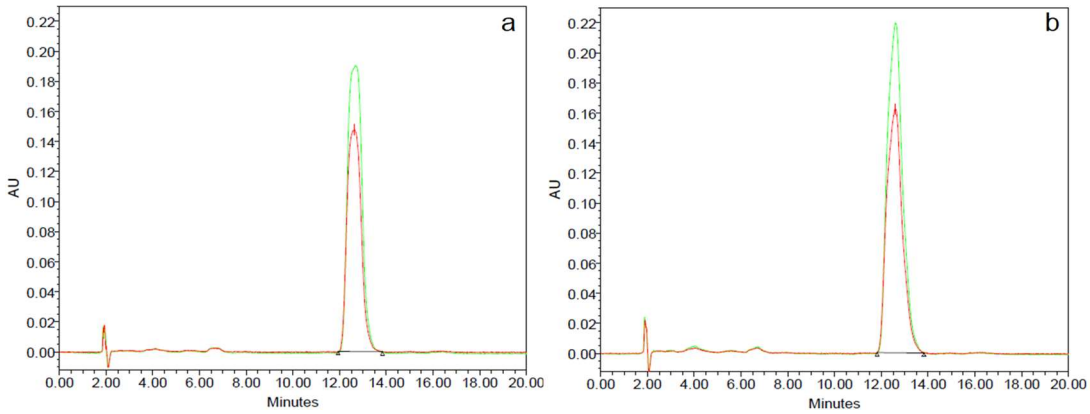

Figure 2: HPLC-UV/Vis chromatograms at 470 nm of acetone extracts from untreated (red curve) and PEF treated (5 kV/cm; 5 kJ/kg, green curve) tomato peels at 20°C (a) and 50°C (b) of extraction temperature.

4. Conclusions

The results obtained in this work demonstrated that PEF pre-treatment coupled with moderate extraction temperatures can improve the extractability of carotenoids from tomato peels achieved after SB of tomato fruits, which is an industrial unit operation typically used for peel removal. In particular, the electroporation effect induced by the electrical treatment with a relatively low energy consumption (5 kJ/kg) enabled to furtherly enhance the degree of cell disintegration induced by the previous SB treatment, thus intensifying the extractability of carotenoid compounds. Interestingly, the application of a PEF pre-treatment allowed reducing the extraction temperature without affecting the extraction yield. Moreover, HPLC analyses revealed that lycopene was the most predominant carotenoid in the peel extracts, hence responsible for their antioxidant power. In a future work, the effects of PEF pre-treatment on the intensification of the extractability of carotenoids from peels derived from industrial processing of tomatoes will be investigated in order to validate the results of the present research on a real food processing matrix.

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