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The use of deep eutectic solvents and pulsed electric field as a pre-treatment for ultrasound-assisted extraction of bioactive compounds from tomato peel

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ABSTRACT

Tomato peel waste is a valuable source of bioactive compounds such as lycopene, β-carotene and polyphenols, which can be sustainably extracted using deep eutectic solvents (DES). In this study, pulsed electric field (PEF) pre-treatment combined with ultrasound-assisted extraction was applied to optimize recovery. The highest lycopene concentration (282.55 mg/mL) and antioxidant activity (50.24 %) were obtained at 6 min extraction and 2 μs pulse duration. Under the same conditions, total polyphenols reached 0.995 mg/mL, while a slightly higher value (1.025 mg/mL) with similar antioxidant activity (50.27 %) was obtained in treatment of 9 min with 0.5 μs pulse duration. The maximum β-carotene content (50.65 mg/mL) was obtained in treatment of 6 min and 2 μs pulse duration. Antioxidant activity strongly correlated with lycopene and polyphenol contents, while colour parameters were influenced by the concentration of bioactive compounds, particularly carotenoids, and by treatment duration. Increasing treatment time and pulse duration raised the specific energy input but caused partial degradation of carotenoids and polyphenols, confirming that mild PEF conditions enhance extraction efficiency while preserving compound stability. These results demonstrate the potential of DES combined with optimized PEF parameters as a sustainable approach for producing tomato-based products with improved nutritional and functional properties.

1. Introduction

The tomato ranks as one of the most popular vegetable crop in the Mediterranean diet. The beneficial effects of regular tomato consumption are generally attributed to the high content of carotenoids. The tomato fruit is also rich in other bioactive compounds with highlighted antioxidant and health-promoting properties such as polyphenols, flavonoids, ascorbic acid and vitamin E (Frusciante et al., 2007). Lycopene, the dominant carotenoid in tomatoes, is one of the most important antioxidants for human health (Rao & Agarwal, 1999). Lycopene has a characteristic chemical structure with eleven conjugated double bonds in the polyene chain, usually in the *all*-trans configuration which isomerise into one of the 72 possible *cis*-isomers, mainly 5-, 9-, 13- or 15-*cis*, found in plants and plasma. The isomerisation of *trans*-lycopene is usually related to the processing and storage conditions during the experiment, i.e. exposure to radiation, light, high temperatures and air. The strong antioxidant activity and the characteristic bright reddish colour are a consequence of the chemical structure of lycopene (Bramley, 2000; Holloway, Yang, Paganga, Rice-Evans, & Bramley, 2000). Besides lycopene, the most abundant carotenoid in tomatoes is beta-carotene, a carotenoid responsible for the orange colour, strong antioxidant activity and the properties important for human health (Jalali-Jivan, Fathi-Achachlouei, Ahmadi-Gavlighi, & Jafari, 2021).

The tomato processing produces a large amounts of tomato waste and by-products, especially seeds, and peels, which are rich in bioactive compounds. Compared to tomato juice and pellets, tomato by-products contain a higher concentration of lycopene and beta-carotene. Conventional extraction processes, such as maceration, boiling, grinding, Soxhlet, reflux, etc. are generally used to extract of bioactive components from waste. The conventional extraction methods are time-

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consuming and require large quantities of organic solvents (Xi, Li, & Fan, 2021). In recent years, the development and use of non-thermal and non-destructive technologies for the extraction of bioactive components is in the focus of numerous research studies (Ates et al., 2025). The use of novel technologies in combination with environmentally friendly, so-called "green" solvents ensures food safety and quality (Cano-Lamadrid & Artés-Hernández, 2022; Marinaccio et al., 2024). These new extraction technologies include supercritical fluid extraction (SFE), ultrasound-assisted extraction (UAE), ultra-high-pressure extraction (UHPE), enzyme-assisted extraction (EAE), microwave-assisted extraction (MWAE) and pulsed electric field (PEF)-assisted extraction (Chatzimitakos et al., 2023). PEF technology has great potential for use in the food and pharmaceutical industries due to its economic efficiency and minimisation of environmental impact. PEF technology uses short duration high-voltage electric pulses to increase the membrane permeability and ensure mass transfer (Raso et al., 2016). The pore formation (electroporation) increases the permeability of the cell membrane and enhances the diffusion of bioactive components through the membrane (Xi et al., 2021). Depending on the treatment parameters (external electric field, pulse duration and treatment time) and the cell properties, electroporation of the cell membrane can be reversible or irreversible (Ranjha et al., 2021). Electroporation of the cell membrane causes a reduction in the total number of microorganisms and a change in the colour, flavour and concentration of bioactives and antioxidants in food (Miklavčič, 2012). The electric field strength is the most important parameter for determining the degree of extraction as it directly affects the physical properties of the desirable compound, such as diffusivity and solubility. The electric field strength of 12 to 45 kV/cm is sufficient to extract the valuable components from the food, but the PEF intensity depends on the properties of food (Ranjha et al., 2021). The effectiveness of PEF treatment in the extraction of bioactive compounds depends not only on the processing parameters, but also on the type of solvent, the composition of the sample, the size of the bioactive component and the position in the cell cytoplasm. PEF-assisted extraction ensures higher yields and shorter extraction times, while reducing the need for conventional organic solvents that are often associated with environmental and safety concerns as described in details in review paper of Gidado et al. (Gidado, Gunny, & Alnashef, 2025). The entire process can be carried out at ambient temperature, which preserves the structure and increases the concentration of bioactive components (Jin & Yin, 2010; Xi et al., 2021).

Ultrasonic assisted extraction (UAE) is considered a clean and environmentally friendly method capable of recovering target metabolites by disrupting the cell membrane through the transmission of ultrasonic pressure waves and the phenomenon of cavitation (Luengo, Condón-Abanto, Alvarez, & Raso, 2014). The phenomenon of acoustic cavitation consists of the formation, growth and collapse of microbubbles in a liquid exposed to high-frequency sound waves (Feng & Yang, 2010). The mechanical effects of ultrasound improve the isolation of the desired compounds from their matrices by disrupting the cellular tissue and allowing better penetration of the solvent into the cellular material (Luengo, Condón-Abanto, et al., 2014). Ultrasonic treatments, like PEF treatments, can be performed at low temperatures, which is desirable in the extraction of thermolabile bioactive components that are degraded during processing (Tzima, Brunton, Lyng, Frontuto, & Rai, 2021). High productivity, yield and selectivity, shorter processing time, improved quality and reduced chemical and physical hazards are some of the main advantages of ultrasonic extraction compared to conventional extraction methods (Chemat, Huma, & Khan, 2011). The combination of ultrasound and PEF could lead to improved efficiency of extraction of nutrients from agro-industrial waste.

As already mentioned, the conventional organic solvents used for the extraction of bioactive compounds are unsafe and need to be vaporised, which is why research is focusing on the use of green solvents. The development of technologies for the synthesis of green substances is mainly concerned with the production of more environmentally friendly solvents used in the recovery of bioactive components from food waste (Vasyliev, Lyudmyla, Hladun, Skiba, & Vorobyova, 2022). Nowadays, green solvents known as Deep Eutectic Solvents (DESs) and Natural Deep Eutectic Solvents (NADESs) are used for extraction. DESs and NADESs are liquids with unique properties formed by the combination of two or more solid components in an appropriate molar ratio, consisting of hydrogen bond acceptor (HBA) and hydrogen bond donor (HBD), which interact mainly through hydrogen bonds and create a liquid product (Socas-Rodríguez, Torres-Cornejo, Alvarez-Rivera, & Mendiola, 2021). The presence of asymmetric components in DES and NADES reduces the energy and causes instability of the ionic structure, resulting in a lower melting point, and also makes it more viscous than water and conventional organic solvents (Jablonsky, Skulcová, Malvis, & Sima, 2018). The wide range of extraction capacity, thermal and chemical stability, polarity, component variability and non-volatility are the advantages of DES and NADES compared to conventional organic solvents. The main advantage of DES compared to conventional organic solvents is the ability to customise the components of the deep eutectic solvent system to the requirements of the bioactives during synthesis to achieve higher extraction yields (Vasyliev et al., 2022). The wide range of valuable properties of DES and NADES offers great potential for their use in the environmentally friendly extraction of bioactive compounds from waste. DES and NADES are eco-friendly, exhibit low or almost zero toxicity and ensure higher product yields compared to conventional extraction solvents. In a previous study on bioactive compounds from orange peels (Viñas-Ospino et al., 2023a, Viñas-Ospino, Panić et al., 2023b), hydrophobic DES using DL-menthol:camphor (Me:Cam) in a molar ratio of 1:1 proved to be one of the most promising extraction solvents and stabilisers for carotenoids. Due to its low solubility in water, relatively low viscosity compared to other DES and high thermal stability, the combination of menthol and camphor in DES promises a higher extraction yield of water-insoluble carotenoids, in this case lycopene and beta-carotene (Illoussamen et al., 2025). In addition, the orange peel extract from Me:Cam showed the highest permeability for carotenoids, improved the stability of the extracted compounds during a 60-day storage and also preserved the antioxidant activity.

From the current research results it can be concluded that tomato peels can be regarded as a valuable source of important bioactive compounds. The combination of DES and PEF pre-treatment with ultrasound is favourable for the extraction of bioactive compounds from tomato peels. Essentially, PEF pre-treatment electroporates the cell membranes to increase the yield of bioactive compounds contained in the tomato peels and enhance the effect of UAE.

This combination increases the extraction yield and supports more environmentally friendly extraction methods that use environmentally friendly solvents and reduce energy consumption. To the best of our knowledge and the available literature, this type of research and the presented methodological approach have not yet been investigated. Therefore, the aim of the present study is to investigate the combined efficiency of PEF, UAE and DES for the extraction of bioactive compounds from tomato peels in terms of total carotenoid and polyphenol content, antioxidant activity and colour parameters in order to develop a sustainable, environmentally friendly processing method for the recovery of natural antioxidants from agricultural waste.

2. Materials and methods

2.1. Chemicals

Methanol and methyl tert-butyl ether (MTBE) were purchased from

VWR (Vienna, Austria) and Folin-Ciocalteu reagent from Merck (New Jersey, USA). Sodium carbonate was purchased from Kemika (Zagreb, Croatia) while L (+) - ascorbic acid from Gram-mol (Zagreb, Croatia). Gallic acid was obtained from Thermo Scientific™ Chemicals (Waltham, Massachusetts, USA) and camphor from Thermo Fisher (Kandel, Germany). Menthol, 2,2-diphenil-1-picrylhydrazyl (DPPH·), lycopene (≥85.0 % HPLC) and beta-carotene (≥95 % HPLC) standards were purchased from Sigma (St. Louis, MO, USA). All chemicals used in analyses were HPLC grade.

2.2. Tomato plants

Fresh tomato plants (*Solanum lycopersicum L.*, plum shaped cultivar) were obtained from the Croatian organic farm "Vrtni centar Baković" (Holus d.o.o., Sveti Filip i Jakov, Croatia). The peeling method of tomatoes was carried out by hot and cold break after which peels were separated using a sharp knife. The separated peels were dried in a dryer at 70 °C until a constant mass was achieved and grounded into powder in a mill. The dried and ground peels were stored in a desiccator at 25 °C until analysis. The moisture content of the dried peels was in the range of 10–15 % wet basis, ensuring their stability for storage and suitability for subsequent extraction.

2.3. DES preparation

The deep eutectic solvent (DES), consisting of menthol and camphor (Me:Cam) in a molar ratio of 1:1, was prepared according to the method described by (Viñas-Ospino et al., 2023a). Menthol (hydrogen bond donor, HBD) and camphor (hydrogen bond acceptor, HBA), whose chemical structures are shown in Fig. 1, were accurately weighed and added together in the indicated molar ratio to a flask without adding water. The mixture was stirred continuously for 2 h at 50 °C until a clear and homogeneous liquid was formed. The prepared DES was then sealed and stored at room temperature until further use.

2.4. Extraction of bioactive compounds using ultrasound with PEF as pre-treatment

Extraction of bioactive compounds from tomato peel was performed using high-frequency ultrasound and PEF as pre-treatment. All PEF treatments were performed in a PEF reactor HVG60/1 PEF (Impel d.o.o., Croatia). The reactor consisted of two stainless steel electrodes with a diameter of 68 mm and a distance of 1 cm, previously described in (Bebek Markovinović et al., 2022). Tomato peel powder in an amount of 2 g was added to the reactor and filled up with 25 mL (Me:Cam) solution. The PEF treatments were performed according to the methods previously described in Markić et al. (Markić et al., 2025) and the parameters

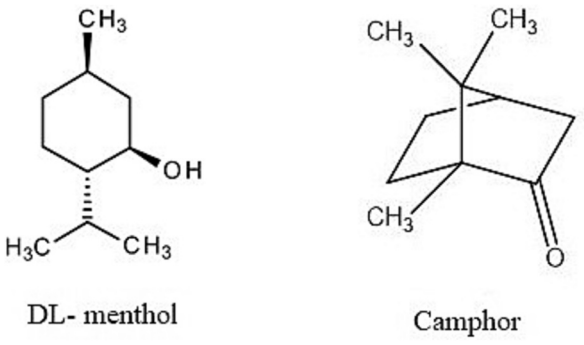

Fig. 1. Chemical structure of menthol (hydrogen bond donor, HBD) and camphor (hydrogen bond acceptor, HBA) used for DES preparation.

Table 1
The specific input energy of PEF during the pre-treatment process.

Sample Identifier	Time (min)	Pulse duration (μs)	Energy per pulse (J)	Specific energy input (J/mL)
1	6	0.5	0.005	5.59
2	6	1.25	0.013	13.99
3	6	2	0.021	22.38
4	9	0.5	0.005	8.39
5	9	1.25	0.013	20.98
6	9	2	0.021	33.57
7	12	0.5	0.005	11.19
8	12	1.25	0.013	27.97
9	12	2	0.021	44.75

listed in Table 1. The energy analysis required in the study is based on the energy value per pulse and the specific input energy according to the equations below (Raso et al., 2016):

- calculation of the energy per pulse (J/pulse) of the PEF

$$W_{pulse} = U \cdot I \cdot \tau \quad (1)$$

- calculation of the pulse-specific energy or pulse energy density (kJ/mL/pulse)

$$W_{spec} = \frac{N}{V} W_{pulse} \quad (2)$$

where N is the total number of pulses (dimensionless), V is the total volume of the sample filled into the PEF treatment chamber (mL), τ is the duration of each pulse, U is the PEF output voltage, and I is the electric current applied to the sample. In our case, since the application of the PEF was applied in a static homogeneous solid–liquid extraction, where the same pulse type and pulse duration are applied for each treatment group, the values of $U(t)$ and $I(t)$ were considered constant.

Using the above equation, it was estimated that the specific energy input for all PEF-assisted extractions ranged from 5.59 to 44.75 J/mL as shown in Table 1.

After the PEF pre-treatment, the samples were sonicated in a Bandelin Sonorex Digiplus DL 255H (Bandelin electronic, Germany) ultrasonic bath with a maximum power of 640 W and a frequency of 35 kHz for 30 min at 25 °C. The samples were shaken for 10 min at 3000 rpm and centrifuged for 10 min at 10000 rpm. The supernatant was then separated and used to determine the total carotenoid and polyphenolic content, antioxidant activity and colour. In order to investigate the effects of PEF pre-treatment on the physico-chemical properties of tomato peels in DES, a control sample was kept for each experiment. The control sample, i.e. sample identifier 0, was not PEF pre-treated, but ultrasonicated in bath for 30 min under the same conditions as all PEF-treated samples.

2.5. Total carotenoid content (TCC)

The total carotenoid content of tomato peel extracts was determined by high-pressure liquid chromatography-HPLC (Agilent Technologies, USA) on a non-polar C30 column (Luna 250 mm × 4.6 mm, 5 μm, 100 Å, Phenomenex, USA) at 35 °C. The mobile phases were methanol: MTBE: mQ water (90:3:7 (v/v) (solvent A) and methanol: MTBE (10:90 (v/v) (solvent B) at a constant flow rate of 0.8 mL/min. For the separation of carotenoids, gradient chromatography was performed as follows: 0 min 100 % A, 0 % B; 20 min 70 % A, 30 % B; 35 min 50 % A, 50 % B; 45 min 20 % A, 80 % B; 50 min 0 % A, 100 % B; 52 min 100 % A, 0 % B. Before injection, 500 μL of the sample was diluted in 1500 μL of solvent B; the

injection volume was 10 μL. The carotenoids were detected and identified using a DAD at a wavelength of 280 nm, and the UV spectra were recorded in the range of 190–400 nm. The carotenoids were quantified by comparing their retention time and UV spectra with those of the commercial standards - lycopene (25–500 mg/mL) and beta-carotene (50–1000 mg/mL).

2.6. Total polyphenolic content (TPC)

The total polyphenolic content (TPC) of extracts was determined by Folin-Ciocalteu's (FC) assay. The measurements were performed with spectrophotometer Secomam UviLine 9400 (Secomam Groupe Aqualabo, France). Briefly, 2 mL of mQ water and 300 μL of the extracts were mixed with 200 μL of FC reagent and incubated at room temperature in the dark for 3 min. In addition, 1 mL of 20 % (*w/v*) sodium carbonate was added and the mixture was incubated at room temperature in the dark for 2 h, then the absorbance was read at 765 nm. The calibration curve of gallic acid (0.06–0.3 mg/mL) was used to calculate the concentration of polyphenols in the samples, and the results are expressed in mg/ml GA equivalents.

2.7. Antioxidant activity

The antioxidant activity of samples was estimated using EPR-DPPH· assay. The volume of 40 μL of sample was mixed with 960 μL of DPPH solution (15 mM in 96 % ethanol). The blank sample was prepared by mixing the same volume of DPPH· and 96 % ethanol solution instead of extract. Bruker Magnettech ESR5000 spectrometer (Bruker BioSpin, Germany) was used for recording X-band ESR spectra at room temperature. Measurements were conducted with the following set of parameters: magnetic field from 331 to 344 mT; modulation amplitude 0.2 mT, microwave frequency 100 kHz; microwave power 10 mT. Antioxidant activity corresponds to the decrease in the EPR signal intensity. Therefore, the antioxidant activity of the tomato peel extracts is expressed as a percentage, which is calculated using the following formula:

$$\%\text{reduction of DPPH} = \frac{A_b - A_s}{A_b} \times 100 \quad (3)$$

Where the A_b is the amplitude of the blank sample and A_s is the amplitude of the tomato peel extracts. A calibration curve for ascorbic acid (0.005–0.2 mg/mL) was established to calculate the concentration of antioxidants in tomato peel extracts and the results are expressed as ascorbic acid equivalents (AAE).

2.8. Colour determination

The colour parameters were determined immediately after extraction using the Spectroquant Prove 300 spectrophotometer (Merck Darmstadt, Germany). To determine the colour, the 2580 CIELAB D65/2° method was used, in which the value L^* (lightness) indicates the ratio of white to black, the value a^* the ratio of red to green and the value b^* (yellowness index) the ratio of yellow to blue (Pathare, Opara, & Al-Said, 2013). A volume of 5 mL was added to each sample in quartz cuvettes and recorded at 25 °C. The blank sample consisted of an acetone-hexane solution (4:6 (*v*/v)).

2.9. Statistical analysis

Measurements were performed in triplicate and results are presented as the mean of three consecutive measurements ± standard deviation (SD) unless otherwise stated. A one-way analysis of variance (ANOVA) was performed to determine possible differences in the results with $p \leq 0.05$. The means of the treatments were separated using an unequal Tukey's test for honestly significant differences (HSD) with $p \leq 0.05$. A Pearson correlation test was performed between all measured values.

Table 2
The content of lycopene and beta-carotene in DES extracts from tomato peels. The results are expressed as mean values ± SD. Within each column, statistically significant differences ($p < 0.05$) are indicated by different superscript letters.

Sample Identifier	Lycopene (mg/mL)	Beta carotene (mg/mL)
0	202.95 ± 4.17^{cd}	28.00 ± 1.70^{a}
1	195.15 ± 9.12^{cd}	44.70 ± 1.98^{bc}
2	263.20 ± 7.78^{ef}	50.65 ± 0.92^{c}
3	282.55 ± 11.67^{f}	44.05 ± 0.49^{bc}
4	186.45 ± 1.63^{bc}	45.10 ± 0.14^{bc}
5	228.90 ± 17.69^{de}	47.25 ± 2.05^{bc}
6	204.90 ± 12.30^{cd}	41.15 ± 3.32^{b}
7	148.10 ± 7.21^{ab}	42.95 ± 3.46^{bc}
8	143.00 ± 5.37^{a}	42.30 ± 1.27^{b}
9	228.35 ± 18.74^{de}	44.20 ± 2.40^{bc}

Statistical analyses were performed using Systat v.13.2.01 (Grafiti LLC, Palo Alto, US).

3. Results and discussion

3.1. Total carotenoid content

The total carotenoid content of tomato peel extracts, expressed as the content of lycopene and beta-carotene as the predominant carotenoids, is shown in Table 2. Statistical analysis revealed a significant impact of PEF pre-treatment on the extraction of carotenoids in tomato peel extracts. The highest lycopene concentration was obtained in sample 3, which was treated with 6 min and 2 μs pulse duration, while the lowest concentration was obtained after a treatment of 12 min and 1.25 μs pulse duration (sample 8). Although the control sample, i.e. sample identifier 0 exhibited a higher lycopene concentration compared to samples 1, 4, 7, and 8, this can be explained by the specific PEF conditions applied. At lower to intermediate energy inputs (5–12 J/mL), the treatment was not sufficient to enhance lycopene release, while the combination of prolonged treatment time and pulse duration may have promoted structural changes leading to isomerization and partial degradation of lycopene rather than improved extraction. Such effects have been previously reported for carotenoids exposed to intense

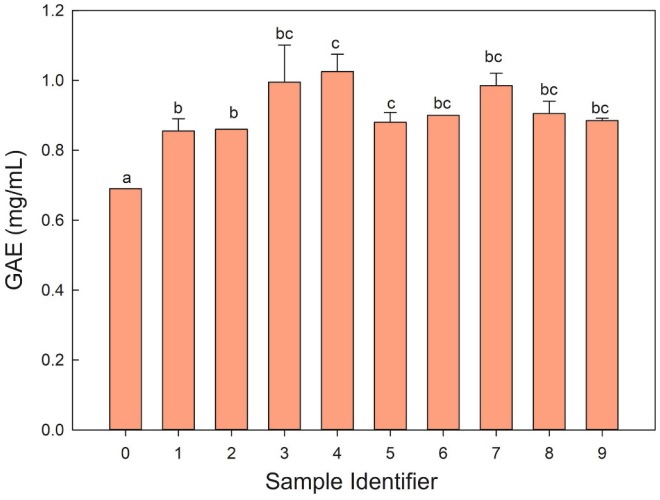

Fig. 2. Total polyphenolic content expressed as mg GAE (gallic acid equivalent)/mL of tomato peel extracts in DES. Statistically significant differences within the bars ($p < 0.05$) are indicated by different superscript letters.

processing conditions (Pataro, Carullo, & Ferrari, 2019; Shi & Le Maguer, 2000). In contrast, samples exposed to higher energy inputs, e.g., 2 (13.99 J/mL) and 3 (22.38 J/mL), showed significantly increased lycopene levels (263.20 and 282.55 mg/mL), supporting the observation that the effect of PEF is strongly dependent on treatment intensity and duration rather than being uniformly positive.

3.2. Total polyphenolic content

The impact of PEF pre-treatment parameters on total polyphenolic content is shown in Fig. 2. PEF pre-treatment significantly improved the extraction of polyphenols from tomato peels, with treatments of 6 min and 2 μs pulse duration and 9 min and 0.5 μs extracting the highest concentration of polyphenols. The lowest concentration was found in the control sample. Of all treated samples, a treatment of 6 min and 0.5 μs extracted the lowest concentration of polyphenols.

3.3. Antioxidant activity

The antioxidant activity of the DES extracts from tomato peels was determined using the 2,2-diphenyl-1-picrylhydrazyl radical, i.e. the EPR-DPPH· assay (Table 3). The EPR measurement is based on the ability of antioxidants to reduce the initial concentration of the free radical DPPH· and change its deep purple colour to yellow (Brand-Williams, Cuvelier, & Berset, 1995). The antioxidant activity depends on the concentration of bioactive compounds in the tomato peel extracts. The pigments coexisting with the extracts may affect the spectrophotometric evaluation in the DPPH· test, which is not the case with the EPR measurements. The use of EPR spectroscopy eliminates the pigment-associated limitation of the DPPH assay, as EPR only detects species with unpaired electrons (Yeo & Shahidi, 2019). The highest antioxidant activity was obtained in samples 3 (50.24 %) and 4 (50.27 %) which were treated in time of 6 min and 2 μs and 9 min and 0.5 μs pulse duration. The lowest antioxidant activity (27.19 %) was observed in the control and samples 1 and 2 which were treated in time of 6 min and 0.5 and 1.25 μs pulse duration.

Table 3
Scavenging effect (%) on DPPH· radical of in DES extracts of tomato peel. No statistically significant differences were found between treatments (ANOVA, $p > 0.05$).

Sample Identifier	% reduction of DPPH	Ascorbic acid equivalents (mg/mL)
0	27.19 ± 16.16	0.11 ± 0.03
1	27.19 ± 2.93	0.11 ± 0.01
2	27.19 ± 7.71	0.11 ± 0.03
3	50.24 ± 7.29	0.15 ± 0.01
4	50.27 ± 6.25	0.11 ± 0.01
5	42.01 ± 0.50	0.14 ± 0.00
6	45.41 ± 11.40	0.14 ± 0.02
7	45.61 ± 1.52	0.14 ± 0.00
8	37.86 ± 5.93	0.13 ± 0.01
9	48.33 ± 3.75	0.15 ± 0.01

Table 4
CIELab parameters of tomato peel extracts: L^* indicated the lightness, a^* the red/green coordinate, and b^* the yellow/blue. Higher values of a^* are redder, while higher values of b^* are more yellow rather than blue. Results are expressed as means ± SD. Within each column, statistically significant differences ($p < 0.05$) are indicated by different superscript letters.

Sample Identifier	L^*	a^*	b^*
0	67.66 ± 2.10^{b}	36.07 ± 1.14^{a}	121.89 ± 3.29^{ab}
1	63.71 ± 0.90^{ab}	44.40 ± 2.38^{ab}	118.59 ± 0.08^{ab}
2	63.06 ± 0.76^{ab}	44.90 ± 2.38^{b}	117.21 ± 1.61^{ab}
3	68.01 ± 1.59^{b}	45.65 ± 2.63^{b}	124.57 ± 1.65^{b}
4	63.11 ± 0.67^{ab}	45.52 ± 3.01^{b}	117.36 ± 1.59^{ab}
5	64.12 ± 1.61^{ab}	44.56 ± 2.26^{ab}	118.68 ± 2.78^{ab}
6	64.54 ± 0.41^{ab}	43.76 ± 2.42^{ab}	119.10 ± 1.17^{ab}
7	61.50 ± 0.79^{a}	44.17 ± 1.74^{ab}	114.75 ± 1.57^{a}
8	62.15 ± 0.02^{a}	44.22 ± 1.99^{ab}	115.68 ± 0.39^{a}
9	63.41 ± 1.17^{ab}	44.89 ± 2.68^{b}	117.74 ± 2.23^{ab}

3.4. Colour determination

The colour parameters of the DES tomato peel extracts were determined using the CIELab method as follows: L^* indicates the lightness, a^* indicates the red/green coordinate and b^* the yellow/blue coordinate, as shown in Table 4.

3.5. Pearson correlation analysis and discussion

A Pearson correlation analysis was performed between the PEF pre-treatment parameters (treatment time and pulse duration) and the content of bioactive compounds and the colour values, as shown in Table 5. In addition, a correlation analysis was performed to verify the magnitude and direction of the correlation between lycopene, beta-carotene, total polyphenol content and the colourimetric data of the CIELab colour system and antioxidant activity (Table 6).

The pulse duration of the PEF pre-treatment has very weak or no correlation with any of determined values, while the time of the PEF pre-treatment correlates significantly with most of the values. Accordingly, the specific energy input, which is linearly proportional to both quantities, i.e. the pulse and the treatment duration, correlates with these values. An increase in specific energy input improved the extraction of lycopene and beta-carotene in tomato peel extracts in the study by Pataro et al. (Pataro, Carullo, Falcone, & Ferrari, 2020). The results in Fig. 2 show an increase and a decrease in lycopene and beta-carotene concentrations depending on the specific energy input. A longer pulse and a longer treatment duration increase the specific energy input, but not the concentration of carotenoids in all treated samples. A higher specific energy input decreased the carotenoid concentration, probably due to electroporation and degradation of the lycopene and beta-carotene structure. Electric field strength, pulse duration and applied voltage are the key parameters of PEF treatment that determine whether reversible or irreversible electroporation occurs. The application of a high electric field leads to the formation of free radicals (reactive oxygen and nitrogen species) and their reaction with bioactive components and the degradation of their chemical structure. Numerous studies have investigated the effects of PEF treatment parameters on the extraction of carotenoids from tomato peel, juice and seeds, focussing on electric field strength, pulse and treatment duration (Belgheisi et al., 2024; González-Casado, Martín-Belloso, Elez-Martínez, & Soliva-Fortuny, 2018; Luengo, Álvarez, & Raso, 2014). In the study by Belgheisi et al. (2024), the maximum increase in carotenoid content was obtained in treatments of 1.25–5 μs, in which the concentration of *trans*-lycopene was increased by 27 % and that of 9-, 13-, 15-*cis*-lycopene by 94 %, 140 % and 33 %, respectively, confirming the effects of PEF treatment parameters on the isomerisation of *trans*-lycopene into one or more *cis*-forms. In this study, quantification and the qualification of lycopene were determined using the calibration curve of the standard solution for *trans*-lycopene, and the *cis*-forms were not analysed, therefore a significant decrease in *trans*-lycopene concentration was observed after PEF treatments with longer treatment and pulse duration. According to the results shown in Table 2, higher lycopene and beta-carotene content were extracted with shorter treatment time and longer pulse duration, which can be attributed to higher electroporation, better solvent diffusion into the intracellular matrix and better solute transport. The better solvent diffusion can be explained by the higher extraction rate in the initial phase, when the solvent rapidly penetrates the intracellular matrix due to the high concentration gradient between the solid and liquid phases. The extraction rate gradually decreases over time as the concentration of bioactive

Table 5
Correlation matrix of PEF pre-treatment parameters and concentration of bioactives and colourimetric parameters of tomato peel extracts.

	Lycopene	Beta-carotene	TPC	AAE	L^*	a^*	b^*
Pulse duration	0.4907	−0.0450	−0.1974	−0.2822	0.3421	0.0057	0.3931
Treatment time	−0.2633	0.7628*	0.7650*	0.7555*	−0.8470**	0.8450**	−0.7539**

* significant at the 0.05 level.
** significant at 0.01 level.

Table 6
Correlation matrix of concentration of bioactives, antioxidant activity and CIELab colour parameters of tomato peel extracts.

	Lycopene	Beta-carotene	TPC	AA	L^*	a	b
Lycopene	1						
Beta-carotene	0.2302	1					
TPC	−0.0128	0.8783***	1				
AA	−0.4173	0.5712	0.6928*	1			
L*	0.4519	0.9608***	−0.7361**	−0.6328*	1		
a*	0.1441	−0.7162**	0.8623***	0.7015*	−0.6960*	1	
b*	0.5816*	−0.5498	−0.5927*	−0.5217	0.9727***	−0.5153	1

* significant at the 0.05 level.
** significant at 0.01 level.
*** significant at 0.001 level.

components in the intracellular matrix decreases until almost an equilibrium state is achieved (Poojary & Passamonti, 2015). The parameters of PEF treatment play a crucial role in improving membrane permeability and should be carefully selected to improve extraction pathways and increase the concentration of bioactive components in tomato peel extracts. A statistically important factor in the extraction process of the desired compounds is the choice of the type of solvent that penetrates into the cells and is responsible for the diffusion of the bioactive compounds (Kyriakoudi, Tsiouras, & Mourtzinos, 2022).

Conventional organic solvents such as n-hexane, ethyl acetate, methanol, acetone, etc. are widely used in traditional solvent extraction. Recently, there have been efforts to reduce the use of organic solvents in extraction due to their environmental impact and toxicity and the desire for safer and more sustainable alternatives. Vlachoudi et al. (Vlachoudi, Chatzimitakos, Athanasiadis, Bozinou, & Lalas, 2023) investigated the effects of different DES on the extraction of carotenoids from tomatoes compared to conventional organic solvents (n-hexane, ethyl acetate, acetone). The total carotenoid content was increased after all DES extractions compared to conventional solvents, with menthol-based DESs providing the best results. To improve the extraction of carotenoids from tomato peels and ensure higher yields, a suitable DES was prepared, physico-chemically characterised and tested for its efficacy in improving the extraction of lycopene and beta-carotene from tomato peels. For this purpose, menthol:camphor DES was used in a molar ratio of 1:1. Menthol and camphor were selected due to their hydrophobic character, natural origin, low toxicity and excellent solvating capacity for non-polar compounds, making them particularly suitable for the isolation of lipophilic carotenoids, as described in Vinas Ospino et al. (Viñas-Ospino et al., 2023b). Despite their efficiency, the viscosity of DES is high, which is why various studies have shown that the addition of different co-solvents (ethanol, water) can improve the extractability of bioactive compounds. The viscosity of DES can be a limiting factor in the extraction process, which can reduce the diffusion of the bioactive compounds, affecting the extraction yield and leading to an increase in extraction time.

The concentration of polyphenols in tomato peel extracts depends on the duration of treatment and the pulse duration. The TPC decreased with increasing the treatment duration, while a short treatment and pulse duration increased the TPC in the tomato peel extracts. As with the total carotenoid content, the most important parameter for the extraction of polyphenols is the specific energy input, which determines the electroporation of the cell membrane. The destruction of the cell membrane is directly proportional to the frequency and pulse duration of the applied high-voltage pulse (Athanasiadis et al., 2023). The use of high specific energy input in PEF treatments can activate the activity of enzymes such as polyphenol oxidase, polyphenol peroxidase, lipoxygenase and pectin methyl esterase, which degrade phenolic compounds (Belgheisi et al., 2024; Vallverdú-Queralt et al., 2012). Polyphenols are bioactive compounds that are mainly contained in the vacuoles of tomato cells. Therefore, two separate barriers must be overcome to extract polyphenols: the vacuolar membrane and the cell membrane (Ferrari, Donsì, & Pataro, 2011). The use of more intense PEF conditions is required to induce sustained electroporation of both membranes, which increases the TPC content in tomato peel extracts. For the above reasons, the PEF protocol must be carefully selected and optimized to improve the extraction of polyphenols without causing irreversible electroporation. In addition to the processing parameters, the use of the adequate solvent type also plays an important role in the extraction. The solvents traditionally used for the extraction of polyphenols, such as ethanol, methanol, acetone, ethyl acetate and their mixtures, are not compatible with ecological and sustainable development due to disadvantages such as high cost, difficult synthesis techniques, high toxicity and poor recoverability of bioactive compounds (Zhou, Fakayode, & Li, 2023). From a chemical point of view, TPC should be increased by using DES, as its composition, chemical nature and physical properties better fulfil the extraction requirements. In the study by Vorobyova et al. it was found that choline chloride: lactic acid (1:2 M ratio) and choline chloride: 1,2-propanediol are the most suitable for the extraction of polyphenolic compounds (Vorobyova et al., 2023). In this study, menthol:camphor was used, which is primarily intended for the extraction of lipophilic bioactive compounds such as carotenoids.

The results of antioxidant activity of tomato peel extracts are related to concentration of carotenoids and polyphenols. The increase in the carotenoid and polyphenolic content leads to an increase in the antioxidant activity of the tomato peel samples. The highest concentration of lycopene was 282.55 mg/mL in sample 3 with an antioxidant activity of 50.24 % and in sample 4 with concentration of polyphenols of 1.025 g/mL GAE with an antioxidant activity of 50.27 %. Lycopene and beta-carotene are the most important carotenoids in tomato peel, with lycopene having a stronger antioxidant effect. Several studies investigated the polyphenolic content of tomatoes and reported that quercetin is the most abundant and important antioxidant among the polyphenolic compounds (George, Kaur, Khurdiya, & Kapoor, 2004; Slimestad, Fossen, & Verheul, 2008; Vadez-Morales, Espinosa-Alonso, Espinoza-Torres, Delgado-Vargas, & Medina-Godoy, 2014). Despite the strength of the antioxidant properties of the compounds mentioned, the

antioxidant activity of tomato peel extracts cannot be directly associated to a specific bioactive compound in the extract, but is related to the mutual interactions of all antioxidants and other bioactive components in the extract (ĆetkoviĆ et al., 2012).

The results show that the brightest colour in the tomato peel extracts occurred in the control sample (sample without PEF pre-treatment), while the lowest brightness was measured in sample 7, which was treated for 12 min and with a pulse duration of 0.5 μs. Positive values in all measurements of the a^* and b^* parameters of colour indicate red and yellow rather than green and blue colour. Statistical analysis showed that the time of PEF pre-treatment duration had the greatest influence on the colour of the tomato peel extracts (Table 4 and Table 5). The pulse duration of the PEF pre-treatment correlates only very week or not at all with the colour values. The parameter a^* shows a strong positive correlation with the duration of the PEF pre-treatment ($r = 0.8450$, $p = 0.001$), while b^* shows a strong negative correlation ($r = -0.7539$, $p = 0.005$). A strong positive correlation was found between L^* and beta-carotene ($r = 0.9608$, $p = 0.000$) and L^* and b^* value ($r = 0.9727$, $p = 0.000$); TPC and beta-carotene ($r = 0.8783$, $p = 0.000$), antioxidant activity and a^* value ($r = 0.7015$, $p = 0.011$). A moderate positive correlation was observed for lycopene and b^* value ($r = 0.5816$, $p = 0.047$) and antioxidant activity and TPC ($r = 0.6928$, $p = 0.013$). A strong negative correlation was found for TPC and L^* ($r = -0.7361$, $p = 0.006$) and beta-carotene and a^* ($r = -0.7162$, $p = 0.009$), while a moderate negative correlation was found between antioxidant activity and L^* ($r = -0.6328$, $p = 0.012$) and between L^* and a^* value ($r = -0.6960$, $p = 0.120$). A positive correlation was observed between lycopene and L^* ($r = 0.4519$, $p = 0.140$) and a negative correlation between lycopene and antioxidant activity ($r = -0.4173$, $p = 0.177$). On the other hand, a very weak correlation was observed between lycopene and a^* value. In the study by Lazzarini et al. (Lazzarini et al., 2022), the lycopene and beta-carotene content were negatively correlated with the L^* value, i.e. samples with a higher lycopene and beta-carotene concentration had a lower L^* value. In this study, the determination of colour can only be related to the concentration of natural pigments in the tomato peel for some samples. Sample 3, which was treated for 6 min and a pulse duration of 2 μs, contained the highest concentration of lycopene and consequently had the reddest colour of all treated samples. The same effect was observed in samples 2 and 9. Among all treated samples, the 12-min treatments with pulse durations of 0.5 and 1.25 μs showed the lowest lycopene concentrations (148.10 mg/mL for the 0.5 μs pulse and 143.00 mg/mL for the 1.25 μs pulse). These same samples also exhibited the lowest a* values (44.17 for the 0.5 μs pulse and 44.22 for the 1.25 μs pulse), which are directly related to the red component of the colour space. Consequently, the reduction in lycopene content was accompanied by a decrease in a* values, resulting in a brighter and less intense red colouration. This confirms the expected correlation between lycopene concentration and CIELAB a* values, as reported in previous studies (Brandt, Pék, Barna, Lugasi, & Helyes, 2006; Pandurangaiah, Sadashiva, Shivashankar, Rao, & Ravishankar, 2020) on tomato carotenoids, where lower carotenoid levels correspond to lighter red colouration. Compared to the control sample, samples 1, 4, 7 and 8 had a lower concentration of lycopene, so a lighter colour than the control sample was expected, but the results showed the opposite effect, these samples had a darker colour than the control sample, which contained a higher concentration of lycopene. The concentration of beta-carotene in the mentioned samples (1, 4, 7 and 8) was higher than in the control sample, which underlines the influence of beta-carotene on the formation of the red colour of tomato peel. In addition to the lycopene and beta-carotene content, the colour changes of the tomato peel extracts can be associated with the decompartmentation process, in which a reaction occurs between enzymes and their substrates as a result of the electroporation-induced migration of cell contents (González-Casado et al., 2018). The colour differences between samples are an important parameter for the visual representation of the product and should be taken into account in the formulation of food, cosmetics and pharmaceutical products. Since this combination of treatment and DES (Me:Cam) was used for the first time for the extraction of tomato peels, there is no literature data about study under same conditions for comparison.

4. Conclusions

The extraction of bioactive components from tomato peels was performed by PEF pre-treatment in ultrasound-assisted extraction. Comparison with the control sample, which was treated with ultrasound alone, showed that PEF pre-treatment increased the concentration of bioactive compounds and enhanced the antioxidant activity of the extracts, indicating that PEF-assisted ultrasound extraction can improve extraction efficiency and the quality of the recovered compounds. PEF pre-treatment also reduced solvent consumption and preserved the quality of the extracted compounds. The effect of PEF pre-treatment was confirmed by a statistical analysis in which the highest content of carotenoids and polyphenols in tomato peel extracts was extracted with a treatment of 6 min and a pulse duration of 2 μs. The antioxidant activity of the extracted samples depends on the concentration of bioactive compounds in the tomato peel extracts. The highest reduction in DPPH signal intensity, i.e. the highest antioxidant activity, was obtained in the sample extracted with the above-mentioned treatment of 6 min and 2 μs, as it contained the highest concentration of lycopene and total polyphenols, which are known as strong antioxidants.

The menthol:camphor DES was used in the PEF-assisted extraction of bioactive compounds from tomato peels. The use of DES in the extraction of carotenoids and polyphenols from tomato peels proved to be very successful. The limiting factor in DES extraction is the viscosity of the DES, which hinders the diffusion of the bioactive components and affects the extraction yield, which in combination with the application of a high specific energy input explains the decrease in the concentration of bioactive components in some tomato peel samples.

PEF and ultrasound-assisted extraction are emerging technologies in food processing that allow more efficient isolation of bioactive compounds, especially when combined with targeted, non-toxic solvents such as DESs. The present study demonstrates that the combination of these innovative techniques can enhance extraction efficiency while preserving the nutritional quality and safety of tomato peel extracts. However, up to our best knowledge, as this is the first application of this kind of multistage approach, further research is required to directly compare its performance with individual extraction techniques and to optimize the protocol for maximum product yield and consistent quality. Such investigations will be essential to assess the industrial applicability and potential advantages of the combined method.

CRediT authorship contribution statement

Franka Markić: Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Manuela Panić:** Writing – review & editing, Methodology, Investigation, Formal analysis, Data curation. **Višnja Stulić:** Writing – review & editing, Methodology, Investigation, Formal analysis, Data curation. **Sanda Pleslić:** Writing – review & editing, Visualization, Formal analysis, Data curation. **Tomislava Vukušić Pavičić:** Writing – review & editing, Validation, Supervision, Methodology, Investigation, Conceptualization. **Nadica Maltar-Strmečki:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization.

Ethical statement

The authors declare that the research presented does not involve any animal or human study.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

The datasets generated during the current study are available from the corresponding authors.

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