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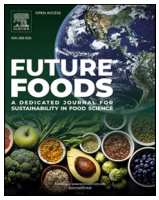

Improving the extraction of tomato seed oil and the retention of bioactive substances using pulsed electric field technology

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ABSTRACT

Tomato seeds are by-products of tomato processing and are rich in important bioactive compounds like carotenoids, polyphenols, phytosterols and fatty acids. The aim of this work was to provide insight into bioactive profile and key physicochemical properties of tomato seed oil (TSO) by using a pulsed electric field (PEF) as a pre-extraction process. Influence of PEF treatment parameters on quality of TSO is reflected in concentration of extracted bioactive compounds, antioxidant activity and oxidative stability of TSO. HPLC and GC analysis revealed that lycopene is the major carotenoid with an average concentration of 565.54 mg/mL and linoleic fatty acid is predominant unsaturated fatty acid in TSO. Results obtained in this work indicate that the PEF treatment parameters of 9 min and 0.5 μs provide the best result in extraction and isolation of bioactive components. Due to the high concentration of bioactive compounds, TSO has strong antioxidant activity, which also plays important role in oxidative stability of the oil. The resistance to free radical formation at high temperatures describes the oxidative stability of TSO, which was investigated by EPR spectroscopy and DSC. This study provides important information about the composition and properties of tomato seed oil, which is crucial for future industrial production and consumption.

1. Introduction

The generation of by-products (waste) is one of the biggest economic and environmental problems in food processing. The plan for a bio-based economy aims to minimize waste production and promote the integration of nutrients recovered from waste into the market (Giannelos et al., 2005; Szabo et al., 2021). The tomato is one of the most important industrial vegetable crops and the main source of antioxidants and important bioactive nutrients in the human diet (Sogi et al., 2005). Every year, millions of tons of waste are produced during the processing of tomatoes. Depending on the peeling process, the tomato industry produces three types of by-products: peels, seeds and tomato pomace-defined as a mixture of peels, seeds and fibers (Szabo et al., 2021). All these by-products are rich in carotenoids, polyphenols, dietary fiber, polyunsaturated fatty acids and other compounds that are desirable for human nutrition due to their antioxidant and therapeutic properties (Ninčević Grassino et al., 2020; Silva and Jorge, 2014). Choosing an appropriate method for the extraction of bioactive compounds from tomato by-products can be challenging due to their different physicochemical properties (Velusamy et al., 2023). Novel, non-thermal technologies minimize the use of organic solvents and shorten the duration of the extraction process. One such method is the pulsed electric field (PEF), which increases the permeability of the cell membrane by electroporation. An increase in mass transfer and solvent diffusion through the cell membrane without the use of heat and excessive amounts of solvents makes PEF a desirable method for the pre-extraction process compared to conventional extraction methods (Andreou et al., 2020).

In PEF technology, high-voltage electrical pulses, usually of short duration, are used to increase the permeability of the membrane and ensure the transport of substances. Pore formation is referred to as electroporation, which can be both irreversible and reversible and is responsible for the diffusion of bioactive components through the cell membrane (Xi et al., 2021). The strength of the electrical field is the

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primary parameter that determines the degree of the extraction process, as it has a direct influence on the physical properties of the investigated compounds. The extraction of bioactive components depends not only on the parameters of the PEF treatment, but also on the type of plant material, the physico-chemical properties of the bioactive compounds, their size and position in the cell cytoplasm (Ranjha et al., 2021). PEF technology has a good potential to be used as a pre-extraction method, as it allows a higher yield of products, shortens extraction time and reduces the consumption of excessive amounts of organic solvents (Jin and Yin, 2010).

Tomato seeds have a good potential to be used as a raw material for the production of vegetable oil, as they have an oil content of 20–36 %. Tomato seed oil (TSO) is a yellow-red liquid with a characteristic odour reminiscent of tomato fruit under ambient conditions. In addition to the industrial production of biodiesel, lubricants and paints, attempts are being made to expand the utilization of TSO to the food, pharmaceutical, nutraceutical and cosmetics markets (Giannelos et al., 2005; Sangeetha et al., 2023). TSOs are an important source of essential fatty acids, which play an important physiological role in the human diet, as they cannot be synthesized in the human organism and have therapeutic effects on health (Vidrih et al., 2010). The high content of polyunsaturated fatty acids and the strong antioxidant activity of TSO prevent atherosclerosis, dilated blood vessels, thrombosis and cholesterol (Sangeetha et al., 2023). On the other hand, due to their chemical nature, these compounds are susceptible to oxidative degradation, which can cause oxidative stress in the human body (Sun et al., 2011).

Oxidation of lipids determines the quality and shelf life and leads to a deterioration of the oil during storage. According to Shao et al. the rate of lipid oxidation is highly dependent on exposure to oxygen, light and temperature during oil storage (Shao et al., 2015). The lipid oxidation process is accompanied by chemical reactions in which reactive oxygen and nitrogen species (ROS and RNS) are formed in three main steps: initiation, propagation and termination (Cui et al., 2017).

Among the many methods used to determine the oxidative stability of foodstuffs, the Rancimat, DSC and EPR methods are the most commonly used in oil research. Most of the methods used to determine the oxidative stability of oils are non-specific and lead to large variations in the results. Electron paramagnetic resonance (EPR) or electron spin resonance (ESR) provides an accurate insight into the reactions that occur during the lipid peroxidation process (Naik et al., 2014). Free radicals are formed during lipid oxidation, making the EPR method suitable for the detailed study of the lipid peroxidation process due to its ability to qualitatively and quantitatively detect species with unpaired electrons (Roman et al., 2010). Free radicals generated during the oxidation process are highly reactive, short-lived and can rarely be detected by direct EPR measurements. To overcome this problem, the spin-trap method is used for EPR measurements. Spin traps are unstable diamagnetic compounds that form a more stable product, the so-called spin adduct, when reacting with free radicals, which is detectable by EPR (Cui et al., 2017). Among the numerous spin traps, N-tert-butyl-α-phenylnitrone (PBN) is commonly used to study lipid peroxidation due to its lipophilic properties and high reactivity (Roman et al., 2010). Previous research reports indicate that PBN is not very stable in some systems under mild conditions and is rapidly degraded under extreme thermal conditions (180 °C) (Janzen et al., 1990; Vicente et al., 1998). Therefore, the stability of spin traps under experimental conditions may be the limiting factor for the study of lipid peroxidation. Oxidation processes and thermal-oxidative deterioration are exothermal processes that are often investigated and detected by differential scanning calorimetry (DSC). Compared to conventional methods, the DSC method is very sensitive and precise, requires a small amount of sample and provides results rapidly (Garcia et al., 2007). The DSC measurements can be performed with a linear temperature increase (dynamic conditions) or at a constant temperature (isothermal conditions). The oxidation medium can be produced by oxygen or air at normal or increased pressure (Ostrowska-Ligeza et al., 2010).

The DSC method has shown good correlation with other methods used in lipid peroxidation studies such as EPR or Rancimat (Cross, 1970; Thomsen et al., 2000). DSC and Rancimat methods usually measure advanced oxidation, called termination, through the production of volatiles during heat release. In contrast, the spin-trapping method of EPR spectroscopy monitors the early stage of the oxidation process, called initiation, by trapping the free radicals produced by the oxidation process (Velasco et al., 2004). To date, only a few new technologies such as supercritical fluid extraction, microwave-assisted extraction and ohmic heat extraction have been described for the extraction of tomato seed oil (Kumar et al., 2023; Sangeetha et al., 2023). As far as we know, PEF pretreatment for tomato seed oil (TSO) extraction and its effects on TSO quality have not been well studied (Ferraz and Silva, 2025). For this reason, the aim of this work was to determine the impact of PEF treatment on the extraction of oil and bioactive compounds (lycopene and beta-carotene) from tomato seeds. The correlation between the bioactive composition of the oil and the main physicochemical properties, such as antioxidant activity and oxidative stability, was investigated using high pressure liquid chromatography (HPLC), gas chromatography (GC), EPR spectroscopy and DSC.

2. Materials and methods

2.1. Chemicals

All solvents used in the study were HPLC grade. Methanol and methyl tert-butyl ether (MTBE) were purchased from VWR (Vienna, Austria), isooctane and ethyl acetate were purchased from Carlo Erba (Cornaredo MI, Italy), while N-hexane, potassium hydroxide, sodium chloride and sodium hydrogen sulphate were purchased from Kemika (Zagreb, Croatia). N-tert-butyl-α-phenylnitrone (PBN), 2,2-diphenil-1-picrylhydrazyl (DPPH·), lycopene (≥85.0 % HPLC) standard and beta-carotene (≥95 % HPLC) standard were obtained from Sigma (St. Louis, MO, USA), while FAME 37 standard was purchased from Supelco (Bellefonte, PA, USA).

2.2. Tomato seeds

Fresh tomato plants (*Solanum lycopersicum L.*, cultivar "Roma") were obtained from the Croatian organic farm "Vrtni centar Baković" (Holus d.o.o., Sveti Filip i Jakov, Croatia). The separation of tomato peels, seeds and pulp was performed by hot and cold break. Tomato peels were removed with a sharp knife and the seeds were separated from the tomato pomace using a sieve. The separated seeds were dried in a dryer at 40 °C until a constant mass was achieved and ground into powder in a mill.

2.3. Tomato seed oil extraction

Tomato seed oil (TSO) extraction was performed using a pulsed electric field (PEF) as a pre-extraction process. All PEF treatments were performed using the HVG60/1 PEF (Impel d.o.o., Zagreb, Croatia) which has been previously described (Markovinovic et al., 2022) and whose schematic representation is shown in Fig. 1. The PEF treatment chamber consists of two stainless steel electrodes with a diameter of 68 mm and a distance of 2.5 cm.

Mass of 6 g of tomato seed powder was added to the reactor and filled with 50 mL of n-hexane solution. The treatments were carried out with square-wave pulses with an applied voltage of 10 kV/cm at a frequency of 150 Hz. Table 1 shows the time and pulse duration of each treatment. The amount of input energy per pulse (J/pulse) of the PEF (Raso et al., 2016) was calculated according to Eq. (1),

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

whereby the actual PEF treatment time, *t*, results from Eq. (2):

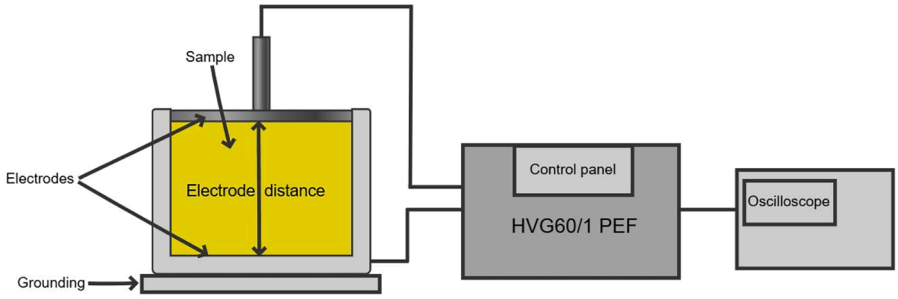

Fig. 1. Schematic representation of the pulsed electric field system.

Table 1
PEF treatment parameters.

Sample ID	Time (min)	Pulse duration (μs)
0	0	0
1	6	0.5
2	6	1.25
3	6	2
4	9	0.5
5	9	1.25
6	9	2
7	12	0.5
8	12	1.25
9	12	2

$$t = \tau \cdot N \tag{2}$$

where τ stands for the pulse duration (μs) and N for the number of pulses (dimensionless).

The pulse-specific energy or pulse energy density (kJ/mL/pulse) is the electrical energy that the treated product receives per pulse, W_{spec} (kJ/mL), for PEF-assisted static extraction was calculated using Eq. (3):

$$W_{spec} = \sum_{0}^{N} \frac{N}{V} \int_{0}^{\tau} U(t) \cdot I(t) dt \tag{3}$$

where N is the total number of pulses (dimensionless), V stands for the total volume of sample (mL) poured into the PEF treatment chamber, τ is the duration of each pulse, $U(t)$ is the output voltage, and $I(t)$ the electric current applied to the sample.

For the application of the PEF in a static homogeneous solid–liquid extraction, where the same pulse type and pulse duration are applied to each treatment group, the values of $U(t)$ and $I(t)$ can be considered constant, so that above function for the pulse-specific energy density is simplified to the following equation for this study (4):

$$W_{spec.} = \frac{N}{V} \cdot U \cdot I \cdot \tau \tag{4}$$

Using the above equation, it was estimated that the specific energy input for all PEF assisted extractions was in the range of 5.594 - 44.755 J/mL.

After PEF treatment, samples were vortexed at 3000 rpm for 10 min and centrifuged at 10 000 rpm for 10 min. To collect more TSO, the supernatant was decanted into a round bottom flask and the precipitate was made up with 50 mL of n-hexane, vortexed and centrifuged. The collected supernatants were mixed and evaporated under reduced pressure using a rotary evaporator (Advantage HL, Heidolph, Germany) to remove the n-hexane and collect the oil. The oil in the control sample was extracted by mixing the powder with n-hexane, vortexing, centrifugation and evaporation. The extracted oil was stored in dark glass vials at room temperature in a desiccator until analysis.

2.4. Total carotenoid content of TSO

The total carotenoid content of TSO was determined by high-pressure liquid chromatography-HPLC (Agilent Technologies, Santa Clara, USA) on a non-polar C30 column (Luna 250 mm × 4.6 mm, 5 μm, 100 Å, Phenomenex, Torrance, USA) heated to 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, 20 mg TSO was diluted in 200 μL of solvent B; the injection volume was 10 μL. Detection and identification of carotenoids were performed using a DAD (diode array detector) at a wavelength of 280 nm, and the UV spectra were recorded in the range of 190–400 nm. The carotenoids were identified and 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).

2.5. Determination of fatty acid composition in TSO

The fatty acid composition of TSO was determined using the Agilent Technologies 6890 N Network Gas Chromatograph (GC) System (Santa Clara, USA). The fatty acid methyl esters (FAMEs) were prepared according to the ISO 12966–2:2017 method (ISO, 2017). FAMEs were separated on a DB-23 capillary column (60 m × 0.25 mm × 0.25 μm) using the GC parameters previously described by Kraljić et al. (2018) with helium as the carrier gas at a constant flow rate of 1.5 mL/min and a split of 60:1. The temperature of the injector was 250 °C and that of the detector 280 °C. The heating rate of the column was 7 °C/min from 60 °C to 220 °C with 17 min retention at final temperature (Kraljic et al., 2018). The fatty acids in the TSO were identified by comparing their retention time with the retention times of commercially available standards. The results are expressed as the percentage of fatty acids in the total amount of fatty acids in the sample.

2.6. Antioxidant activity of TSO

The EPR-DPPH assay was used to determine the antioxidant activity of the TSO samples. A decrease in the EPR signal intensity of the DPPH radical corresponds to the antioxidant activity of the TSO samples. Therefore, the antioxidant activity of the TSO samples is expressed as a percentage, which is calculated using the following formula (5):

$$\%\ \text{reduction of DPPH} = \frac{A_b - A_s}{A_b} 100 \tag{5}$$

Where A_b is the amplitude of the blank sample and A_s is the amplitude of the TSO sample. To determine the decrease in DPPH signal intensity, EPR spectra were recorded immediately after the addition of 980 μL of DPPH· solution (15 mM in ethyl acetate) to 20 μL of the TSO sample and

recorded for 30 min. The blank sample was prepared by mixing the same volume of DPPH· and ethyl acetate solution instead of TSO. X-band ESR spectra were recorded using the Bruker Magnettech ESR5000 spectrometer (Bruker BioSpin, Rheinstetten, Germany) at room temperature. The measurements were performed with the following parameters: magnetic field range from 331 to 344 mT, microwave frequency 100 kHz and modulation amplitude 0.2 mT, microwave power 4.0 mT. A calibration curve for ascorbic acid (0.01–0.3 mg/mL) was established to calculate the concentration of antioxidants in TSO, and the results are expressed as ascorbic acid equivalents (AA).

2.7. Oxidative stability of TSO

Differential scanning calorimetry (DSC) and EPR spectroscopy were used to evaluate the oxidative stability of tomato seed oil. The DSC method was used to determine the induction time of TSO. The measurements were performed with a Differential Scanning Calorimeter 214 Polyma (NETZSCH-Gerätebau GmbH, Selb, Germany) calibrated with high purity indium. The measurements were carried out using a modified method according to Tan et al. (Tan et al., 2002), whereby 4.0 ± 0.3 mg of the samples were weighed into an aluminium dish, sealed with a pin hole lid. The samples were heated to 140 °C under a constant nitrogen flow of 40 mL/min. To achieve temperature stability, the temperature of 140 °C was kept constant for 5 min under nitrogen flow, and the measurement of the induction time started when the nitrogen flow was replaced by an oxygen flow at a rate of 100 mL/min. During the analysis, the inert gas nitrogen flowed through the instrument at a flow rate of 60 mL/min. NETZSCH Proteus Thermal Analysis 8.0.1 software (Netzsch, Selb, Germany) was used to determine the induction time by taking the intersection of the extrapolated baseline and the tangent of the exothermic part of the thermogram.

The oxidative stability monitored by EPR spectroscopy was based on the spin-trapping method using PBN spin trap. 1 g of PBN spin trap was dissolved in the dark in 96 % ethanol solution to a total concentration of 2.5 M. 10 µL of the prepared solution was placed into a glass vial and the ethanol was completely evaporated in a stream of nitrogen, after which 200 µL of TSO was added to the vial. The solution was vortexed for 1 min, placed in an EPR capillary tube and the kinetic series were monitored at 140 °C. The temperature was controlled to within ± 0.1 °C with a TCH04 variable temperature controller (Bruker BioSpin, Germany) using a cold N_2 vapor stream. The EPR parameters were: magnetic field from 330 to 345 mT, sweep 15 mT, modulation amplitude 0.1 mT, microwave frequency 100 kHz, microwave power 20 mW. ESRStudio (Bruker BioSpin, Germany) was used for spectral analysis.

2.8. Statistical analysis

Each measurement was performed in three replicates. Results were expressed as mean ± standard deviation (SD) unless otherwise stated. To assess possible differences between treatments, the experimental data were analysed with a one-way analysis of variance (ANOVA) with a $p \leq 0.05$. Statistical analyses were performed using Systat v.13.2.01 (Grafiti LLC, Palo Alto, USA). Tukey's honestly significant difference test (Turkey HSD) was applied to determine which specific pairs of means were significantly different from each other ($p \leq 0.05$).

3. Results and discussion

3.1. Energy analysis during treatment

The information about the energy requirements during PEF processing can be an important parameter for determining the extraction success. Eqs. (1), (3) and (4) show that the energy per pulse depends on the output voltage, the pulse duration and the current generated by the PEF. In this experiment, the current value is the same for each treatment, which leads to the conclusion that energy input depends only on the pulse duration and the time of the treatment. According to the results in Table 2, the highest specific input power of the PEF was calculated for a treatment with a pulse duration of 2 µs and 12 min. The lowest specific PEF energy was found for a treatment of 6 min and 0.5 µs pulse duration. It can be seen that a longer pulse duration and a longer treatment duration cause an increase in the pulse-specific input energy, while a shorter pulse duration and a shorter treatment duration cause a decrease in the specific input energy. Applying a higher electric field strength increases the energy per pulse and produces high-voltage pulses, which should increase mass transport across the cell membrane, but the overall permeabilization of the cell membrane is tuned to the type of cell. The results of this experiment show the highest value of specific energy of 44.755 J/mL, which is still a low energy for the requirements of extraction of bioactive components. According to Donsi et al. 2010, electric field strengths above 35 kV/cm are suitable for PEF treatment to inactivate microorganisms, and electric field strengths of 1–10 kV/cm are sufficient for the extraction process of important bioactive compounds from agro-industrial materials (Donsì et al. 2010).

3.2. Total carotenoid content

The results of the total carotenoid content in tomato seed oil after PEF treatment are shown in Fig. 2. The statistical analysis shows a significant influence of pulse length and treatment duration on the extraction of total carotenoid content ($p \leq 0.05$) in one sample only. In the other PEF-treated samples and the control sample, statistical analysis revealed no significant difference in lycopene and beta-carotene concentrations. A significant increase in total carotenoid content was observed after PEF treatment at a duration of 9 min and a pulse length of 0.5 µs with a detected lycopene concentration of 1815.434 mg/mL and beta-carotene of 87.159 mg/mL. The lowest lycopene concentration in TSO was obtained after a treatment duration of 12 min and a pulse length of 0.5 µs, which showed no significant difference compared to a control sample. The positive effect of PEF treatment on the extraction of lycopene was only observed in two samples, namely samples 2 (6 min, 1.25 µs) and 4 (9 min, 0.5 µs), which had a higher concentration of lycopene compared to the control sample. For beta-carotene content, only a treatment of 9 min and 0.5 µs led to an increase in total concentration compared to the control sample. The impact of pulse length, treatment duration and pulse-specific energy input are closely related to the total carotenoid content in TSO. A longer treatment duration led to an increase in pulse-specific energy input, which resulted in a greater decrease in the concentration of lycopene and beta-carotene in TSO. For example, among all treatments, the treatment with 9 min and 0.5 µs pulse duration showed the highest lycopene concentration of 1815.434 mg/mL and a low pulse-specific energy input of 8.392 J/mL. The increase in lycopene and beta-carotene content in the extracted TSO was obtained in treatments with shorter treatment and pulse duration and higher pulse-specific energy input (0.5 and 1.25 µs).

In studies by Luengo et al. (2014a) and Belgheisi and coworkers (2024), the increase in electric strength in PEF treatments reduces the carotenoid content in tomato peel extracts through irreversible

Table 2
PEF specific input energy during the treatment process.

Sample ID	Time (min)	Pulse duration (µs)	Energy per pulse (J)	Pulse-specific energy (J/mL)
1	6	0.5	0.005	5.594
2	6	1.25	0.013	13.986
3	6	2	0.021	22.378
4	9	0.5	0.005	8.392
5	9	1.25	0.013	20.979
6	9	2	0.021	33.566
7	12	0.5	0.005	11.189
8	12	1.25	0.013	27.972
9	12	2	0.021	44.755

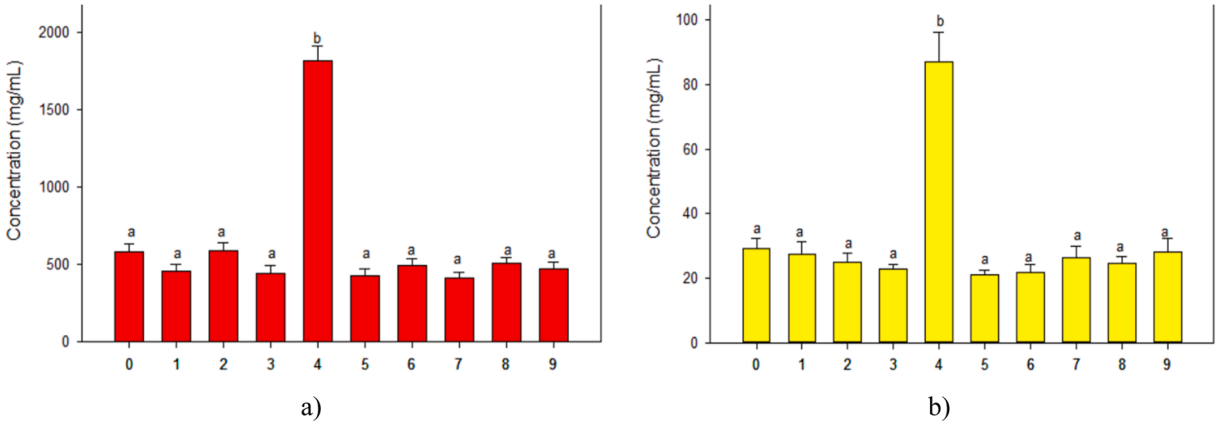

Fig. 2. Total carotenoid content in TSO determined by HPLC after the PEF treatment, a) lycopene concentration b) beta carotene concentration. Results are expressed as means of mean ± standard deviation. Statistically significant differences ($p \leq 0.05$) are indicated by different letters.

electroporation and the production of reactive oxygen species that react with bioactive components and degrade their chemical structure (Belgheisi et al., 2024; Luengo et al., 2014a). According to the studies mentioned above, the increase in carotenoid content occurs at low applied voltage and is associated with the activation of carotenoid metabolic pathways and an increase in extractability from food due to the permeability of the cell membrane. The increase and decrease of lycopene and beta-carotene is related to pore formation (reversible and irreversible) and structural reorganisation induced by PEF treatment (Barba et al., 2015). The release of carotenoids is also limited by several barriers, chromoplasts, cell wall and cell membrane (Schweiggert and Carle, 2017). Therefore, PEF parameters should be carefully selected to enhance membrane permeability, solvent diffusion and mass transfer, and ultimately increase the total carotenoid content. Several studies have reported the effects of PEF treatment parameters on the extraction of carotenoids from tomatoes, focusing on electric field strength, number of pulses and treatment duration (González-Casado et al., 2018; Luengo et al., 2014b). Fig. 2 shows that lycopene is the major carotenoid in tomato seed oil, which is consistent with Eller et al. (Eller et al., 2010). Among the 72 possible isomers of lycopene, the predominant isomer in tomatoes and tomato by-products is the all-*trans*-lycopene isomer (Boileau et al., 2002; Clinton et al., 1996). Exposure to light, high temperatures and oxygen as well as storage conditions are triggers for the isomerization of all-*trans* lycopene to one or more *cis* isomers (Xianquan et al., 2005), mainly 13-*cis* and 5-*cis* (Lambelet et al., 2009). Therefore, the extraction method may have an influence on the conversion of the all-*trans* to the *cis* form of lycopene (Eller et al., 2010). The parameters of the PEF treatment, i.e. pulse length, applied voltage, pulse-specific energy input and treatment duration, are the limiting factors for the isomerization and further determination of the lycopene content in the extracted TSO. In this work, the lycopene concentration in PEF-extracted TSO was calculated using the calibration curve of a standard solution of the all-*trans* isomer of lycopene; the concentration of the *cis* isomers was not investigated. From all this, it can be concluded that the PEF treatment parameters probably influenced the degradation of lycopene and beta carotene. Also, the choice of solvent type has a significant impact on the extraction process of lycopene and beta-carotene, which are lipid-soluble carotenoids (Periago et al., 2004; Strati and Oreopoulou, 2011). It is desirable to choose a solvent that can easily penetrate the membrane pores and ensure diffusion of the oil and high mass transfer.

3.3. Fatty acid composition of TSO

The fatty acid composition of the produced TSO is presented in Table 3. The identified fatty acids in order of retention time are palmitic acid, stearic acid, oleic acid, linoleic acid and linolenic acid. The results in Table 3 show that the most important fatty acid identified in TSO is linoleic fatty acid, which is consistent with the studies of Botineștean et al. (Botineștean et al., 2012). According to Lazos, Tsakins and Lalas (Lazos et al., 1998), the percentage of linoleic acid in the TSO studied was in the range of 50–60 %, palmitic acid was the dominant fatty acid among the saturated fatty acids and linolenic fatty acid had the lowest percentage among the fatty acids mentioned in TSO. The results of this study are consistent with the results of the previously mentioned reports. In addition to the fatty acids listed in Table 3, myristic, pentadecenoic, palmitoleic, margaric, heptadecenoic, arachidic and eicosenoic fatty acids were also detected in the TSO samples in amounts <0.09 %. Based on previous studies (Botineștean et al., 2012; Lazos et al., 1998; Periago et al., 2004) and the results of the fatty acid composition in this work, TSO belongs to the category of oils with linoleic and oleic acid. Due to the fatty acid profile and bioactive content, TSO show good potential for use as food or for some industrial applications such as the pharmaceutical or cosmetic industry.

Table 3
The fatty acid profile of PEF pre-extracted TSO. Results are expressed as means ± SD. Within each column, statistically significant differences ($p \leq 0.05$) are indicated by different superscript letters.

	Percentage fatty acids composition (%) in TSO samples				
Sample ID	Palmitic (16:0)	Stearic (18:0)	Oleic (18:1)	Linoleic (18:2)	Linolenic (18:3)
0	13.054 ± 0.00^{abc}	8.352 ± 0.00^{h}	26.512 ± 0.00^{f}	48.149 ± 0.00^{a}	1.727 ± 0.00^{bc}
1	13.083 ± 0.01^{c}	8.309 ± 0.00^{g}	26.493 ± 0.00^{e}	48.193 ± 0.00^{b}	1.720 ± 0.00^{a}
2	13.049 ± 0.01^{ab}	8.241 ± 0.00^{d}	26.373 ± 0.00^{b}	48.408 ± 0.01^{e}	1.733 ± 0.00^{d}
3	13.060 ± 0.01^{abc}	8.384 ± 0.00^{i}	26.504 ± 0.01^{ef}	48.130 ± 0.00^{a}	1.719 ± 0.00^{a}
4	13.037 ± 0.01^{a}	8.092 ± 0.01^{a}	26.324 ± 0.00^{a}	48.633 ± 0.01^{h}	1.738 ± 0.00^{d}
5	13.079 ± 0.01^{bc}	8.308 ± 0.00^{g}	26.502 ± 0.00^{ef}	48.192 ± 0.01^{b}	1.721 ± 0.00^{a}
6	13.044 ± 0.01^{a}	8.259 ± 0.00^{e}	26.422 ± 0.00^{c}	48.353 ± 0.01^{d}	1.725 ± 0.00^{ab}
7	13.051 ± 0.00^{ab}	8.193 ± 0.00^{c}	26.381 ± 0.01^{b}	48.454 ± 0.01^{f}	1.733 ± 0.00^{cd}
8	13.058 ± 0.00^{abc}	8.281 ± 0.01^{f}	26.462 ± 0.00^{d}	48.282 ± 0.01^{c}	1.723 ± 0.00^{ab}
9	13.049 ± 0.00^{ab}	8.136 ± 0.00^{b}	26.313 ± 0.01^{a}	48.587 ± 0.00^{g}	1.737 ± 0.00^{d}

3.4. Antioxidant activity of TSO

The determination of antioxidant activity using the 2,2-diphenyl-1-picrylhydrazyl radical, i.e. the EPR-DPPH· assay, is a widely used method for evaluating the nutritional value of plant extracts, vegetables, fruits and juices (Magalhaes et al., 2008; Moon and Shibamoto, 2009). The EPR measurement is based on the ability of antioxidants to reduce the initial concentration of the free radical DPPH· and change the color from dark purple to yellow (Brand-Williams et al., 1995). The antioxidant activity of the samples depends on the concentration and bioactive profile of the compounds contained in the analyzed samples. Lycopene and beta-carotene are the main antioxidants in tomatoes and tomato-based products, with lycopene having a stronger antioxidant effect. As our results show, HPLC analysis revealed a higher concentration of lycopene in TSO. The average concentration of lycopene in TSO was 621.94 mg/mL and that of beta-carotene was 31.42 mg/mL. The statistical analysis revealed that only one sample showed a significant difference in the concentration of lycopene and beta-carotene between all the samples tested. Not only the content of lycopene and beta-carotene may have an effect on antioxidant activity, but other bioactive compounds with strong antioxidant properties may also contribute. Eller et al. (Eller et al., 2010) investigated the α-, γ- and δ-tocopherol concentration in TSO and their influence on antioxidant activity. In the study by Toor and Savage (Toor and Savage, 2005), total polyphenols and total flavonoids had a statistically significant effect on the antioxidant activity of tomato seeds. The concentration of total polyphenols was higher than the concentration of total flavonoids, lycopene and ascorbic acid in the seed samples. The results in Table 4 show no significant differences between the TSO samples tested in terms of antioxidant activity. The opposite effect of carotenoid content on antioxidant activity was observed in sample 4, which had a significantly higher concentration of lycopene and beta-carotene, which should have resulted in the highest antioxidant activity, but did not.

3.5. Oxidative stability of TSO

In this work, DSC and EPR spectroscopy were used to determine and compare the oxidative stability of TSO. In EPR measurements, oxidative stability was considered as resistance to free radical formation. Oxidative induction times were determined in the DSC at the intersection of two tangents on the plot of time interval versus amplitude, as shown in Fig. 3a. In our case, due to the shape of the intensity-time curve in the EPR curve, it is not possible to determine the value of the induction time experimentally (Fig. 3b). A similar shape was observed for myrtle seed oil (Fadda et al., 2023). It has also been observed in the literature (Marques et al., 2017) and suggested that the only significant parameter that can be extracted from the kinetic curves representing the EPR intensity of the PBN adduct versus time is the area under the curve (AUC).

All EPR and DSC experiments were performed at 140 °C and the results are presented in Table 5.

Table 4
The antioxidant activity of TSO expressed as percentage reduction of free radical DPPH·, with the concentration of antioxidants expressed as ascorbic acid equivalents (mg/mL). Results are expressed as mean ± SD.

Sample ID	% of DPPH· reduction	AA equivalents (mg/mL)
0	45.08 ± 6.80	0.14 ± 0.02
1	50.42 ± 0.25	0.16 ± 0.00
2	53.79 ± 1.98	0.18 ± 0.01
3	50.80 ± 3.61	0.17 ± 2.43
4	51.77 ± 1.34	0.17 ± 0.00
5	45.10 ± 0.17	0.14 ± 0.00
6	55.03 ± 2.65	0.18 ± 0.01
7	53.02 ± 2.39	0.18 ± 0.00
8	51.20 ± 5.57	0.17 ± 0.01
9	53.25 ± 1.20	0.18 ± 0.00

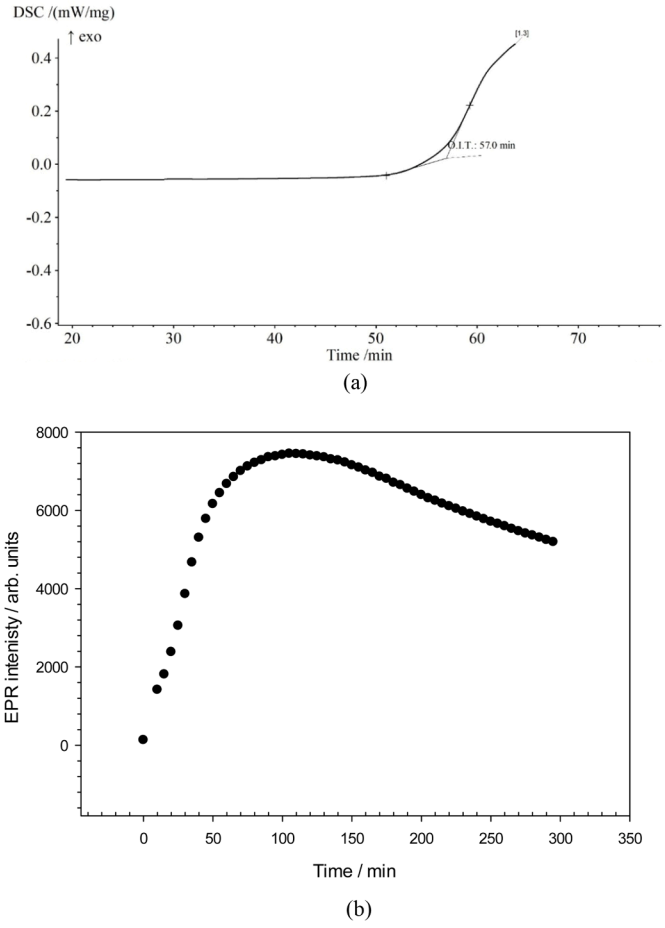

Fig. 3. Determination of the induction time by DSC and EPR spectroscopy on sample No 1: a) Isothermal DSC curve at 140 °C with an induction time of 57 min. b) kinetic curve representing the EPR intensity of the PBN adduct versus time at 140 °C.

Table 5
Induction time (min) at 140 °C determined by DSC and area under curve (AUC) representing the EPR intensity of the PBN adduct versus time determined by EPR method.

Sample ID	Induction time (min) DSC	AUC ($\cdot 10^4$ arb. units) EPR
0	56.10	2.10
1	56.10	1.33
2	55.65	1.35
3	55.80	2.09
4	52.05	1.25
5	55.40	2.04
6	54.65	1.59
7	54.45	1.89
8	54.65	2.95
9	52.85	1.30

The oxidation process of edible oil is an exothermic reaction. The average oxidative stability of TSO determined by induction time using DSC is 54.77 min, the average oxidation stability determined as AUC using EPR spectroscopy is $1.79 \cdot 10^4$ arb. units. The stability of any oil depends on the oil composition mainly on the degree of unsaturation of fatty acids but also on concentration of minor components such as phospholipids, tocols, phenolics, carotenoids, sterols etc. These chemical compounds are also strong antioxidants which help protect oils against oxidative deterioration and hence extend oxidative stability of oil (Naik et al., 2014). The EPR measurements showed greater

fluctuations in the induction time of the samples examined. The explanation for the fluctuations in the results of the EPR-examined TSO samples is strongly related to the working temperature, the sample volume, the experimental procedure and the higher sensitivity of the EPR instrument. The DSC oxidation rate depends only on the availability of oxygen and temperature, in contrast to EPR, which depends on temperature, volume, spin adduct formation, etc. According to Ostrowska-Ligeza and coworkers (2010), use of pure oxygen, in DSC measurements, instead of air may result in lower values of induction time than the actual value (Ostrowska-Ligeza et al., 2010). The lowest AUC, measured by EPR, was obtained in sample 4 treated in time of 9 min with 0.5 μs pulse duration in contrast to sample 8 which was treated in time of 12 min with 1.25 pulse duration and had the highest AUC among all samples. The impact of PEF treatment parameters on oxidative stability determined by DSC is correlated with applied pulse duration, time of the treatment and specific energy input. Longer treatment time and pulse duration caused decrease in oxidative stability of TSO. Since the pulse and treatment duration affect specific energy input, an increase in specific energy input caused decrease in induction time of TSO samples. As was previously mentioned, application of high voltages in PEF treatments causes free radical formation which further react with bioactive compounds disrupting their chemical structure responsible for protection of oxidative deterioration.

The results presented in Table 5 show an impact of PEF treatment parameters on oxidative stability of TSO determined by EPR. The lowest AUC was obtained in sample 4 treated in time of 9 min and pulse duration of 0.5 μs which also had the highest carotenoid content among all treated samples which confirming best resistance to free radical formation. The highest AUC was obtained in sample 7 treated in time of 12 min and 1.25 μs. The treatments with higher pulse duration (1.25 and 2 μs) and longer treatment time caused an increase in AUC time of TSO samples. The combination of long treatment and pulse duration, resulted in higher specific energy input and consequently the lowest induction time of TSO due to free radical formation and degradation of bioactive components in results obtained by DSC and EPR. The stability of the samples before and after the induction phase has an influence on the formation of spin adducts. As already explained, the induction time, i.e. the lag time, cannot be determined experimentally. As Fig. 3b shows, the kinetic curve representing the EPR intensity of the PBN adduct reaches a maximum, and thereafter a decrease in radicals can be seen, confirming that the extracted oil contains antioxidants that reduce the amount of free radicals captured by PBN. The changes in the slopes are related to the formation of spin adducts and changes in the total volume of the sample (Velasco et al., 2004). The spectra of the PBN—OOH spin adduct are presented in Fig. 4. At the beginning of the measurements, the first three spectra show hydrogen splitting, which disappears with increasing signal intensity during time. In the study by Velasco et al. (Velasco et al., 2005) of fish, rapeseed and sunflower oil, all the obtained spectra of the PBN spin adduct consisted of three broad lines, in contrast to the computer simulation of the EPR spectra, which indicated hyperfine splitting from a triplet of doublets with splitting constants of 1.49 and 1.81 mT, respectively. The splitting constants in spectra obtained in this work were determined to be 1.48 and 1.51 mT, respectively. The lack of hydrogen splitting in the EPR spectra was linked by Velasco et al. to the restriction of the rotational mobility of the radicals, which leads to an averaging of the EPR spectra from different orientations of the radicals into a superposition of all individual orientations in a single EPR spectrum. Based on the results, it can be concluded that measurements of oxidative stability of oil at one selected temperature cannot be the base for a comparison of the thermal-oxidative stability of oil. The experiments should be performed at several temperatures in order to determine the oxidative stability as accurately as possible using kinetic models. Except temperature range, further studies on radical formation at different storage conditions, gas flow, PEF treatment parameters, etc. are needed to optimize the parameters for studying the oxidative stability of tomato seed oil.

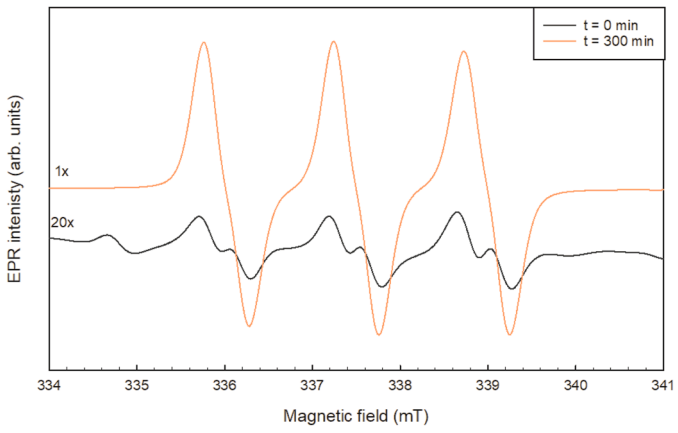

Fig. 4. EPR spectra of PBN—OOH spin adduct: black line at the beginning of the measurement $t = 0$ min, magnified 20 x and red line at the end of the measurement $t = 300$ min.

The formation of free radicals in TSO during the long storage at room temperature affected the oxidative stability and led to different results in EPR and DSC. The treatment of 9 min and 0.5 μs pulse length showed the shortest induction time and consequently the greatest effect of PEF treatment on oxidative stability among all treated samples, which is confirmed by the statistical analysis.

Further studies on radical formation at different temperatures, storage conditions, gas flow, PEF treatment parameters are needed to optimize the parameters for studying the oxidative stability of tomato seed oil.

4. Conclusions

In this study, results of PEF pretreatment for TSO extraction and its effects on TSO quality were presented for the first time. In our experimental design, the treatment with 9 min and a pulse length of 0.5 μs resulted in the highest lycopene and beta-carotene concentrations at a low pulse-specific energy input of 8392 J/mL. In addition, this PEF treatment also showed a positive effect on improving oxidative stability while maintaining the same fatty acid composition and antioxidant activity, which was confirmed by two analytical methods (EPR and DSC), the proven methods for predicting the oxidative stability of vegetable oils. However, further investigation of the bioactive profile of TSO should also include other bioactive components such as polyphenols, flavonoids and other carotenoids, which play an important role in the overall antioxidant activity of TSO. In summary, these preliminary results confirm that the use of PEF as a novel, environmentally friendly method for pre-extraction has a high potential to improve extraction compared to conventional methods, as shown in Fig. 5. Furthermore, our findings provide insight into the bioactive composition and main physico-chemical profile of TSO, which is important for further investigations and optimization of protocols for industrial application. It must also be emphasized that the optimization of pulsed electric field (PEF) treatment protocols is indeed crucial for their effective and efficient application in various fields, especially in food processing and biotechnology.

Ethical statement

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

CRediT authorship contribution statement

Franka Markić: Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis,

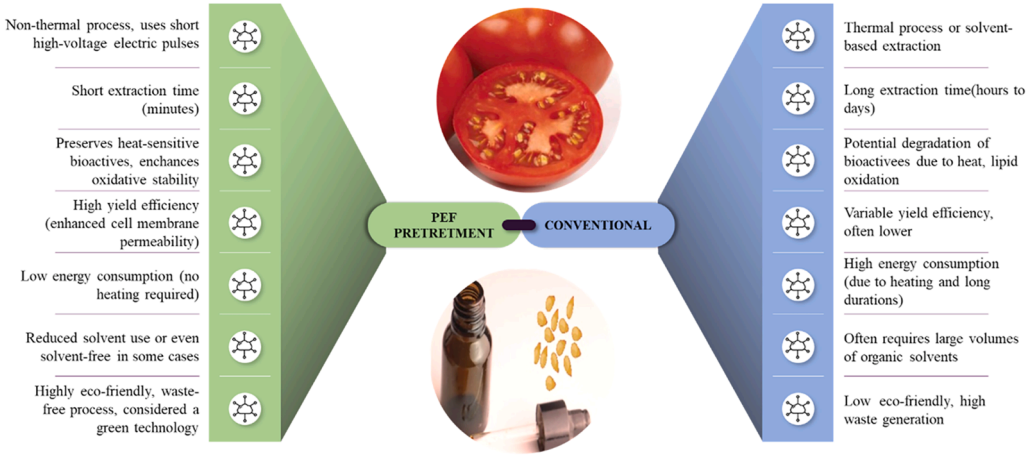

Fig. 5. Advantages of the pulsed electric field (PEF) as a pre-extraction method compared to conventional extraction methods.

Data curation, Conceptualization. **Klara Kraljić:** Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation. **Višnja Stulić:** Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation. **Sanda Pleslić:** Writing – review & editing, Visualization, Formal analysis. **Tomislava Vukušić Pavičić:** Writing – review & editing, Writing – original draft, Visualization, 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.

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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