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Monitoring free radical generation and antioxidant activity in tomato pulp using electron paramagnetic resonance (EPR): effects of ultrasound-assisted bioactive extraction

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ABSTRACT

The effects of ultrasound-assisted processing on bioactive compounds, antioxidant activity, colour parameters, and free radical formation in tomato pulp were investigated. Treatments at different amplitudes and durations were applied to evaluate changes in bioactive compounds and oxidative processes. Ultrasonic processing showed a strong dependence of bioactive compound extraction, antioxidant activity, and radical formation on treatment parameters, with most conditions increasing total phenolic content (TPC) and antioxidant activity, while generally reducing carotenoid levels. TPC reached maximum of 0.380 mg/mL, while antioxidant activity ranged from 56.13% to 78.63% radical inhibition. Lycopene generally decreased under most conditions, with a maximum of 3.036 μg/mL observed under mild treatment, whereas beta-carotene showed minor variations, reaching up to 1.042 μg/mL. Colour parameters (a^* and b^*) were significantly affected by processing conditions and were closely associated with lycopene, while lightness (L^*) remained stable. EPR spin trapping confirmed a balance between sonochemically produced reactive species and scavenging activity of antioxidant compounds released during processing. A complex interaction was observed between bioactive compounds and oxidative behavior: increased TPC contributed to higher antioxidant activity, while lycopene appeared to influence radical levels. Ultrasound processing improved the functional properties of tomato pulp, highlighting ultrasound technology for controlled modification of food quality and oxidative processes, although further optimization is needed.

1. Introduction

Conventional food processing methods typically rely on high temperatures to ensure microbial safety, however, such treatments can lead to the degradation of temperature-sensitive bioactive compounds and cause undesirable changes in texture and organoleptic properties (Hernández-Hernández et al., 2019; Saputra et al., 2026; Zhang et al., 2026). Consequently, the food industry has increasingly turned to non-thermal processing technologies, including pulsed electric field, cold plasma, ultrasound, supercritical carbon dioxide, and high hydrostatic pressure (Al-Sharify et al., 2025; Jadhav et al., 2021; Zhang et al., 2019). These approaches enable the production of safer, higher-quality foods while better preserving nutritional and functional characteristics (Barbhuiya et al., 2021). Moreover, they contribute to sustainability by reducing energy and water consumption, limiting the use of chemical additives, and minimising food losses during processing and storage (Allai et al., 2023; Barbhuiya et al., 2021; Markić et al., 2026; Martín-Belloso et al., 2023).

Among these technologies, ultrasound has emerged as a promising tool due to its ability to enhance mass transfer and improve extraction efficiency (Linares & Rojas, 2022; Mgoma et al., 2025). However, its application is inherently associated with acoustic cavitation, a phenomenon involving the formation, growth, and collapse of microbubbles that generate extreme localised temperature and pressure conditions (Akti & Yildiz, 2025; Singla & Sit, 2021). These conditions promote the homolytic cleavage of water molecules, leading to the formation of

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highly reactive radicals such as hydroxyl (·OH) and hydrogen (·H), as well as secondary oxidising species (Akti & Yildiz, 2025; Ashokkumar, 2015; Trinh et al., 2025). While these reactive species can enhance extraction processes, they may simultaneously induce oxidative degradation of sensitive bioactive compounds, potentially compromising product quality and functionality (Aribi & Hameed, 2025; Chandimali et al., 2025; Menezes et al., 2024). Moreover, the direct detection and characterisation of short-lived radical species in such complex food systems remain analytically challenging, as most conventional methods rely on indirect measurements of stable reaction products rather than the radicals themselves (Lauricella & Tuccio, 2020). This limitation hinders a comprehensive understanding of radical-driven reactions and their impact on bioactive compound stability, creating a critical need to evaluate the balance between radical formation and antioxidant protection in such systems, particularly under varying processing conditions.

Electron paramagnetic resonance (EPR) spectroscopy is a highly sensitive, non-destructive method for the direct detection and quantification of free radicals, enabling detailed assessment of oxidative processes in food systems (Markić et al., 2025; Menezes et al., 2024). It allows identification of radical species based on their unique spectral fingerprints and, through spin-trapping techniques, stabilisation of highly reactive and short-lived reactive oxygen species (ROS) generated during ultrasound-induced cavitation (Rehman et al., 2016; Smoković et al., 2026). This enables qualitative and quantitative analysis of radicals, including their dependence on ultrasound parameters such as power and frequency, with detection limits in the nanomolar range. EPR spin-trapping methods, in which transient radicals react with spin traps to form stable paramagnetic adducts (Lauricella & Tuccio, 2020), are particularly valuable in ultrasound-assisted extraction, as they allow simultaneous monitoring of radical formation and antioxidant activity (Laajimi et al., 2021). Despite the increasing application of ultrasound-assisted extraction, the relationship between ultrasound-induced radical generation and the preservation of antioxidant compounds in tomato systems remains insufficiently understood, particularly due to the limited number of studies directly monitoring free radical formation in ultrasonically processed tomato matrices using EPR spin-trapping approaches. This highlights the importance of EPR as a direct analytical tool for process monitoring and for understanding the balance between oxidative phenomena and bioactive compound stability during ultrasound treatment. While numerous studies have investigated ultrasound-assisted extraction of tomato bioactive compounds, most have primarily focused on extraction efficiency, antioxidant activity, and physicochemical quality parameters, whereas direct monitoring of transient radical species generated during acoustic cavitation in tomato matrices has received considerably less attention (Meena et al., 2024; Rathnakumar et al., 2023; Tang et al., 2024). In this context, the spin trap 5-diisopropoxyphosphoryl-5-methyl-1-pyrroline-N-oxide (DIPPMPO) was selected for EPR analysis due to its high reactivity towards a wide range of radical species and its ability to form relatively stable and long-lived spin adducts in aqueous systems (Cingesar et al., 2025). Compared to conventional nitrones such as 5, 5-dimethyl-1-pyrroline N-oxide (DMPO), DIPPMPO exhibits improved stability of the resulting adducts and higher trapping efficiency, enabling more reliable detection of transient radicals such as hydroxyl (·OH), superoxide ($O_2^{\cdot-}$), peroxyl (ROO·), and alkoxyl (RO·) species under extended acquisition times (Zielonka et al., 2021). Therefore, DIPPMPO-based EPR spin trapping provides a robust approach for monitoring ultrasound-induced radical formation in complex food matrices.

Accordingly, this study aimed to systematically evaluate the impact of ultrasound-assisted extraction on free radical generation with DIPPMPO spin trapping and antioxidant activity in tomato matrices to improve food control during processing and, consequently, food quality, using EPR spectroscopy as the main analytical method. To the best of our knowledge, studies directly combining ultrasound-assisted processing of tomato matrices with EPR spin-trapping analysis for monitoring cavitation-induced radical formation remain very limited. Therefore, this work provides an integrated mechanistic evaluation of the interplay between ultrasound-induced oxidative processes, antioxidant activity, and the stability of major tomato bioactive compounds under different processing conditions. Particular emphasis was placed on evaluating carotenoids and polyphenolic compounds, as well as their contribution to antioxidant activity and radical behaviour. By simultaneously monitoring radical formation and antioxidant responses, this study contributes to a better understanding of oxidative phenomena occurring during ultrasound processing in complex food systems. The obtained results contribute to a better understanding of oxidative phenomena occurring during ultrasound processing and may support future optimization of processing conditions for improved retention of bioactive compounds.

2. Materials and methods

2.1. Chemicals

Methanol and *n*-hexane were supplied by VWR (Vienna, Austria), while the Folin–Ciocalteu reagent was obtained from Merck (Kenilworth, New Jersey, USA). Acetone and sodium carbonate were purchased from Kemika (Zagreb, Croatia). L (+)-ascorbic acid, 96% ethanol, and 30% hydrogen peroxide were acquired from Gram-mol (Zagreb, Croatia). Gallic acid was purchased from Thermo Scientific™ Chemicals (Waltham, Massachusetts, USA). The spin trap 5-diisopropoxyphosphoryl-5-methyl-1-pyrroline N-oxide (DIPPMPO) was purchased from Focus Biomolecules (Plymouth Meeting, PA, USA). Iron(II) chloride tetrahydrate and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were supplied by Sigma (St. Louis, MO, USA). All reagents used were of HPLC grade.

2.2. Tomato pulp preparation

Fresh tomato fruits were obtained from the Croatian eco farm "Vrtni centar Baković", Sveti Filip i Jakov, Croatia. The tomatoes were washed in water at room temperature and separated from peels and seeds using hot (20 s at 100 °C) and cold break (5 min at 5 °C) methods. The remaining tomato flesh was cut into pieces and homogenised using an Omni GLH 850 General Laboratory Homogenizer (Omni International, Inc., Kennesaw, Georgia, USA), then filtered. Tomato pulp samples (20 g) were prepared by mixing 70% supernatant with 26% precipitate. After mixing, the samples were homogenised again for 3 min at 10 000 rpm.

Samples were then ultrasonicated for 1, 3, and 5 min at amplitudes of 30%, 40%, and 50% using a 20 kHz ultrasound generator (Sonoplus HD 4100, Bandelin electronic GmbH & Co. KG, Berlin, Germany) equipped with a 9 mm diameter probe and 135 μm at 100% amplitude with detailed parameters listed in Table 1.

To neutralize the thermal effect of the ultrasound waves, sonication was performed under temperature-controlled conditions using an ice-water bath. The temperature of the samples was measured

Table 1
Experimental conditions during ultrasound treatment.

Amplitude (%)	Ultrasound intensity (W/cm^2)	Treatment time (min)	Acoustic Power Density, APD (J/g)
30	47.17	1	90
		3	270
		5	450
40	62.89	1	120
		3	360
		5	600
50	78.62	1	150
		3	450
		5	750

immediately after each ultrasound treatment and was found not to exceed 40 °C under any experimental condition. This procedure ensured that the primary effect of the treatment was cavitation rather than thermal effects, with temperatures remaining below the threshold for lycopene isomerisation (approximately 40 °C) (Murakami et al., 2018).

The control sample (ultrasound untreated sample), hereafter referred to as ctrl, was prepared in the same manner as the previously described samples but was not subjected to ultrasonication.

pH was determined using a Multiparameter handheld Liquiline Mobile CML18 device with a digital pH sensor Memosens CPL51E-106/0 (Gerling, Germany). Orion™ pH Buffer solutions (pH 4.01, 7.00, and 10.01) were used for instrument calibration. The pH remained constant throughout the measurements, ranging from 4.50 to 4.55.

2.3. Total polyphenolic content (TPC)

Total polyphenolic content (TPC) in the tomato pulp was determined using the Folin-Ciocalteu (FC) method. A volume of 2 mL Milli-Q water was mixed with 100 μL of sample and 200 μL of FC reagent. The mixture was vortexed, then 1 mL of 20% (w/w) sodium carbonate was added after 3 min. The blank sample contained 100 μL Milli-Q water instead of sample. Prepared samples were incubated in the dark for 2 h at room temperature, after which absorbance at 765 nm was recorded using a UV-VIS spectrophotometer (ONDA TOUCH UV-21, Giorgio Bormac s.r.l., Carpi, Italy). The total polyphenolic content was calculated using a gallic acid calibration curve (0.01–0.3 mg/mL), and results are expressed as gallic acid equivalents (GAE mg/mL).

2.4. Total carotenoid content

The main carotenoids, lycopene and beta-carotene, in tomato pulp were extracted using acetone: *n*-hexane solution. After ultrasound treatment, a mass of 2 g was weighed in Falcon tubes (50 mL) and filled up with 25 mL of acetone:*n*-hexane solution (4:6, v/v). Carotenoids were extracted by mixing the solution for 10 min at 3000 rpm using a vortex V-1 Plus (Biosan, Riga, Latvia), and then centrifuged with a Thermo Scientific Heraeus Multifuge 3S-R Refrigerated Centrifuge (Waltham, Massachusetts, USA) for 10 min at 6000 rpm. The carotenoid content was determined from the supernatant (total volume 3 mL) by measuring absorbance at 663, 645, 505, and 453 nm using a UV-VIS spectrophotometer (ONDA TOUCH UV-21, Giorgio Bormac s.r.l., Carpi, Italy). The blank sample contained 3 mL of 4:6 acetone:*n*-hexane solution (v/v). The concentrations of lycopene and beta-carotene were calculated using the following formula (Nagata & Yamashita, 1992):

$$c_{lyc} = -0.0458\ A_{663} + 0.204\ A_{645} + 0.372\ A_{505} - 0.0806\ A_{453} \quad (1)$$

$$c_{betac} = 0.216\ A_{663} - 1.22\ A_{645} - 0.304\ A_{505} + 0.452\ A_{453} \quad (2)$$

where c_{lyc} denotes lycopene concentration and c_{betac} beta-carotene concentration. A_{663}, A_{645}, A_{505}, and A_{453} are absorbances at 663, 645, 505, and 453 nm, respectively. The concentrations of lycopene and beta-carotene are expressed in μg/mL.

2.5. Colour

Colour determination was performed using the CIELab D65/2° method with a Spectroquant Prove 300 spectrophotometer (Merck, Darmstadt, Germany). The CIELab colour space is defined by three coordinate parameters: L^*, representing lightness from black to white; a^*, indicating chromatic variation from green to red; and b^*, representing chromatic variation from blue to yellow (Bielaszka et al., 2024). All measurements were conducted at room temperature. The total colour change in visual perception between a sample and a reference standard, expressed as a single unitless ΔE numerical value that assesses differences in lightness (ΔL^*), redness/greenness (Δa^*), and yellowness/blueness (Δb^*) has been calculated using the standard formula:

$$\Delta E = \sqrt{\left(L_2^* - L_1^*\right)^2 + \left(a_2^* - a_1^*\right)^2 + \left(b_2^* - b_1^*\right)^2} \quad (3)$$

where $(L_1^* - L_2^*)$ is the difference in L^* (brightness) between two samples, and $\left(a_1^* - a_2^*\right)$ and $\left(b_1^* - b_2^*\right)$ are the differences in the colour coordinates a^* and b^*, respectively.

2.6. Antioxidant activity

The antioxidant activity of tomato pulp samples was determined using the EPR-DPPH method. A volume of 950 μL of 15 mM DPPH solution (in 96% ethanol) was mixed with 50 μL of tomato sample. Samples were incubated for 30 min at room temperature, after which spectra were recorded using the X-band frequency benchtop Bruker Magnettech ESR5000 spectrometer (Bruker BioSpin, Rheinstetten, Germany) with the following EPR parameters: magnetic field 330 - 334 mT, modulation amplitude 0.2 mT, sweep time 30 s, microwave frequency 100 kHz, and microwave power 10 mW. Antioxidant activity in the EPR-DPPH assay corresponds to the change in EPR-DPPH signal intensity. In the presence of antioxidants, the DPPH free radical is scavenged, resulting in a decreased EPR signal. This decrease in EPR signal corresponds to the antioxidant activity of the samples. Antioxidant activity is calculated using the following formula:

$$\%\ DPPH\ reduction = \frac{A_b - A_s}{A_b}\ x\ 100 \quad (4)$$

where: A_b denotes the amplitude of the blank sample and A_s denotes the amplitude of the tomato pulp samples. Antioxidant activity is expressed as the percentage (%) of reduced DPPH radical. The concentration of antioxidants was calculated using an ascorbic acid calibration curve (0.01-0.3 mg/mL) and results are expressed as ascorbic acid equivalents (AAE μg/mL).

2.7. Free radical detection

The detection of free radicals in tomato pulp samples was carried out using the spin trap 5-diisopropoxyphosphoryl-5-methyl-1-pyrroline-N-oxide (DIPPMPO). Immediately after ultrasound treatment, 789 μL of Milli-Q water was mixed with 60 μL of 0.35 mM hydrogen peroxide, 40 μL of 10 mM DIPPMPO, 1 μL of tomato sample, and 40 μL of 0.15 mM iron(II) chloride tetrahydrate. The use of 1 μL of tomato sample was necessary due to the strong matrix effects observed at higher volumes, which resulted in complete suppression of the DIPPMPO signal. EPR spectra were recorded using a Bruker Magnettech ESR5000 spectrometer (Bruker BioSpin, Rheinstetten, Germany) 2 min after the addition of iron(II) chloride, under the following conditions: magnetic field 327–347 mT, modulation amplitude 0.1 mT, sweep time 30 s, modulation frequency 100 kHz, and microwave power 10 mW. Manganese, Mn^{2+} in ZnS (Bruker module E8000137), was used as a field standard to control and calibrate the magnetic field axis.

The same experiment was repeated with Milli-Q water which was ultrasonicated in the same manner as tomato pulp samples. The EPR signal area was used as a measure of the concentration of formed radicals.

2.8. Statistical analysis

Statistical evaluation of the data was carried out using Systat software (version 13.2.01; Grafiti LLC, Palo Alto, California, USA). All analyses were conducted in triplicate, and the results are presented as the mean of three independent measurements ± standard deviation (SD). Statistical differences among samples were assessed by analysis of variance (ANOVA), followed by Tukey's honestly significant difference

(HSD) *post hoc* test, with significance set at $p \leq 0.05$. A correlation matrix was generated to examine relationships between the analysed parameters. Principal component analysis (PCA) was also performed to explore patterns and associations between the evaluated chemical parameters.

3. Results and discussion

3.1. Total polyphenolic content

The results for TPC shown in Fig. 1 indicate that all ultrasound-treated samples exhibited a higher concentration of polyphenolic compounds compared to the control (untreated) sample. The highest concentration was observed after a 5-min treatment at 40% amplitude (0.380 mg/mL), while the lowest among the treated samples was recorded after 1 min at 30% amplitude (0.236 mg/mL) (Table S1, Supplementary materials). It can be observed that longer ultrasound treatments at moderate amplitude (40%) lead to a greater increase in TPC, as do shorter treatments at higher amplitude (Fig. 1).

Multivariate statistical analysis confirmed a significant effect of treatment duration, amplitude, and their interaction on the extraction of TPC in tomato pulp samples ($p < 0.0001$, Table S6, Supplementary materials). Additionally, correlation analysis (Fig. S1, Supplementary materials) revealed a strong positive correlation between TPC and treatment duration ($p = 0.000$, $r = 0.6126$), as well as applied amplitude ($p = 0.001$, $r = 0.5398$). Thus, longer treatments at higher amplitudes increase total polyphenolic content.

Compared to the control sample, the extraction of polyphenolic compounds in treated samples was improved depending on the processing parameters. Polyphenolic compounds are known as secondary metabolites, which are increasingly produced under stress conditions (Xiao et al., 2024). In this context, ultrasound may act as a physical stressor, promoting the release of bound phenolics from the plant matrix and enhancing their extractability. The positive correlation between ultrasound parameters and TPC confirms the effectiveness of acoustic cavitation in enhancing cell disruption and mass transfer, facilitating phenolic release from the plant matrix. Additionally, ultrasound treatment may contribute to the partial inactivation of polyphenol oxidase (Tovar-Pérez et al., 2020), an enzyme responsible for the degradation of phenolic compounds.

These findings are consistent with previous studies reporting that ultrasound improves polyphenol extraction in tomato and other plant matrices (Kumar et al., 2021; Yusoff et al., 2022). The increase in TPC with longer treatment durations and higher amplitudes can be attributed to ultrasonic cavitation, which disrupts cell walls and facilitates the release of bound phenolic compounds (Tovar-Pérez et al., 2020). Similar trends have been observed in other tomato-based studies, where moderate ultrasound intensity and optimized treatment duration significantly improved total polyphenolic content without causing thermal degradation (Solaberrieta et al., 2022). During longer treatments at higher amplitudes, the local temperature of tomato samples increases. Previous studies have demonstrated that increasing extraction temperature can enhance total polyphenolic yields by improving solvent diffusivity, phenolic solubility, and the release of bound phenolics from plant matrices, typically up to an optimal range of 60–80 °C (Antony & Farid, 2022). However, excessive heating may degrade heat-sensitive phenolic compounds, highlighting the need to balance thermal input with extraction efficiency (Dzah et al., 2020).

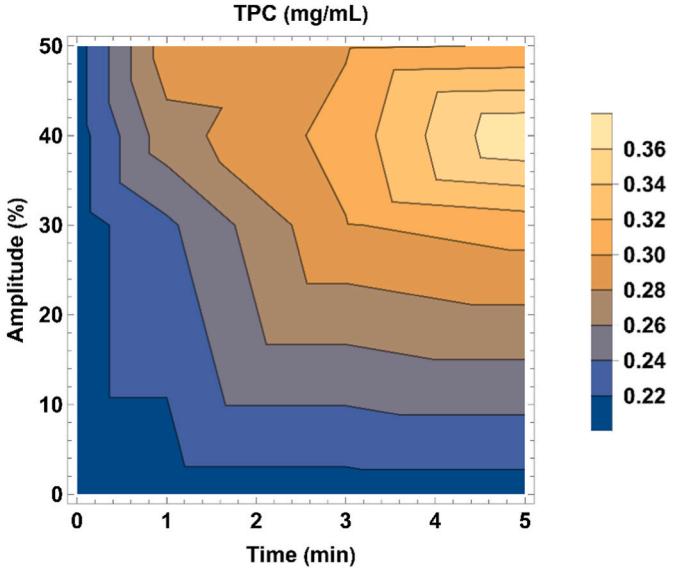

Fig. 1. Contour plot representing the TPC as a function of amplitude and time.

3.2. Total carotenoid content

In this study, the most abundant carotenoids in tomato, lycopene and beta-carotene, were determined as shown in Fig. 2. Statistical analysis showed a significant effect of treatment duration ($p = 0.0003$), amplitude ($p = 0.0001$), and their combination ($p = 0.0004$) on the extraction of lycopene from tomato pulp samples (Table S6, Supplementary materials). Compared to the control sample, a slight decrease was observed. A higher concentration (3.036 μg/mL) of lycopene in the ultrasound-treated sample was found only in the sample treated for 3 min at 40% ultrasound amplitude (Table S2, Supplementary materials). All other samples showed a lower concentration of lycopene than the control (Fig. 2a). The lowest concentration was obtained in the sample treated for 1 min at 40% amplitude (1.773 μg/mL). Correlation analysis (Fig. S1, Supplementary materials) revealed a statistically significant negative correlation ($p = 0.003$, $r = -0.5028$) between lycopene concentration and ultrasound amplitude, as well as TPC ($p = 0.000$, $r = -0.6101$). It can be observed that the application of higher amplitude led to a greater decrease in lycopene concentration in tomato samples compared to lower amplitude combined with longer treatment duration.

Results obtained for beta-carotene, the second most abundant carotenoid in tomato pulp, are shown in Fig. 2b. Statistical analysis showed no significant impact of processing parameters on beta-carotene concentrations in treated samples (Table S6, Supplementary materials), as did correlation analysis. However, most treated samples contained a lower concentration of beta-carotene than the control sample (0.819 μg/mL) (Table S3, Supplementary materials). The highest concentration among all treated samples was observed after treatment for 5 min at 50% amplitude (1.042 μg/mL), and the lowest after treatment for 3 min at 50% amplitude (0.583 μg/mL).

Ultrasound processing parameters showed an opposite effect on carotenoid content in tomato samples compared to conventional processing approaches. Correlation analysis demonstrated a significant negative relationship between lycopene content and ultrasound amplitude, suggesting partial degradation or reduced stability of lycopene under intensified cavitation conditions. Carotenoid degradation is primarily associated with oxidation and isomerisation (Shi et al., 2022). In general, thermal and technological treatments can either decrease or enhance carotenoid concentration depending on processing intensity (Szabo et al., 2022). Prolonged heating, drying, and mechanical disruption often promote degradation, whereas controlled thermal treatments may improve bioavailability through cell structure breakdown (Pathak & Sagar, 2023). These observations indicate that carotenoid stability is highly process-dependent, highlighting the importance of investigating alternative non-thermal technologies such as ultrasound processing (Li et al., 2023). In the present study, ultrasound treatment resulted in the degradation of both lycopene and beta-carotene, with a more pronounced reduction observed for lycopene. This difference can be explained by variations in carotenoid chemical structure and

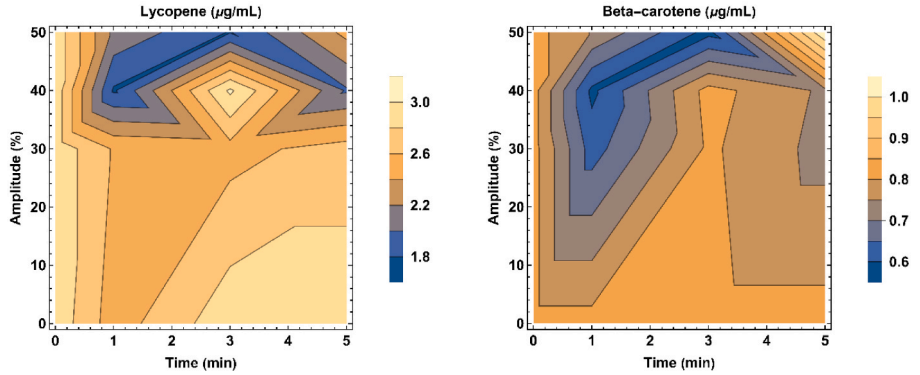

Fig. 2. Contour plot representing a) lycopene (μg/mL) and b) beta-carotene (μg/mL) as a function of amplitude and time.

stability. Lycopene is an acyclic carotenoid composed of 40 carbon atoms with 11 conjugated double bonds, which increases its susceptibility to oxidative degradation and *cis-trans* isomerisation induced by ultrasound-generated cavitation, localized high temperatures, and free radical formation (Jiang et al., 2024; Tufail et al., 2024). In contrast, beta-carotene contains cyclic end groups that provide greater structural stability against oxidation and isomerisation, resulting in comparatively lower degradation (Jiang et al., 2024; Tufail et al., 2024). A strong inverse correlation between lycopene and total phenolic content ($p = 0.000$, $r = -0.6101$) indicates that extraction conditions favouring phenolic release may not be optimal for carotenoid preservation. The absence of significant correlations between beta-carotene and ultrasound parameters suggests higher resistance of this carotenoid to cavitation-induced degradation compared to lycopene. The observed degradation of lycopene (from 2.933 to 1.773 μg/mL) following ultrasound treatment is consistent with previous studies reporting reductions in lycopene content in ultrasound-processed fruit matrices. Similar trends have been reported in recent studies investigating ultrasound processing of tomato-based systems. For example, ultrasound treatment of tomato juice caused a small but statistically significant decrease in lycopene content with increasing ultrasound intensity (Starek et al., 2021). In tomato paste, high-frequency ultrasound pre-treatment was shown to modify bioactive compounds depending on treatment duration and energy input, confirming that ultrasound is not always a mild technology and can influence carotenoid content (Piazza et al., 2022). Review literature on ultrasound processing of fruits and vegetables shows that total lycopene may be reduced in some matrices due to oxidative degradation induced by cavitation and localized high energy conditions, whereas higher sonication intensity or longer treatments can also increase accessibility or bioaccessibility of lycopene, indicating a balance between release and degradation mechanisms that is strongly dependent on processing parameters (Ashraf et al., 2022; Meena et al., 2024; Saini & Keum, 2018). Therefore, the balance between enhanced mass transfer and ultrasound-induced oxidative stress appears to determine the final carotenoid retention in tomato matrices.

The observed changes in bioactive compounds can be explained by the dual role of ultrasound-induced acoustic cavitation. The collapse of cavitation bubbles generates localized high temperatures and pressures, leading to the formation of reactive oxygen species (ROS) as well as intense mechanical forces that disrupt plant cell structures (Mansouri et al., 2020). This dual effect promotes the release of bound phenolic compounds, resulting in increased extractability and higher measured TPC, while simultaneously exposing sensitive carotenoids to oxidative conditions and degradation pathways such as oxidation and isomerisation (Campos-Lozada et al., 2022; Kuvendziev et al., 2024). Therefore, ultrasound processing induces a dynamic balance between enhanced mass transfer and cavitation-driven oxidative stress, which ultimately determines the composition and stability of bioactive compounds in tomato pulp.

3.3. Colour determination

Colour parameters in the CIELab system include three axes, which determine lightness (L^*), red/green colour (a^*), and yellow/blue colour (b^*). All three values, L^*, a^*, and b^*, in ultrasonicated samples are lower than in the control sample (Table 2). Statistical analysis showed no significant impact of treatment duration ($p = 0.343$), amplitude ($p = 0.211$), or the combination of duration and amplitude ($p = 0.365$) on the L^* colour parameter (Table S6, Supplementary materials) In contrast, the a^* and b^* parameters were significantly affected by both the ultrasound processing parameters and their interaction ($p = 0.000$).

Correlation analysis revealed a strong positive correlation between the a^* and b^* values ($p = 0.000$, $r = 0.9503$) (Fig. S1, Supplementary materials), indicating coordinated development of red and yellow colour components. Lycopene content was positively correlated with both a^* ($p = 0.001$, $r = 0.5751$) and b^* ($p = 0.002$, $r = 0.5311$), while beta-carotene showed no significant correlation with a^* or b^*. In contrast, total phenolic content (TPC) exhibited significant negative correlations with a^* ($p = 0.000$, $r = -0.5935$) and b^* ($p = 0.000$, $r = -0.6340$). The results indicate that L^* values correlated negatively with lycopene

Table 2
CIELAB colour parameters (L^*, a^*, b^*) of the tomato pulp samples, where L^* is the vertical axis, a^* is the horizontal axis, and b^* is the depth axis.

	Colour parameters		
L^*	30%	40%	50%
Ctrl		21.5 ± 2.12	
1	24.0 ± 2.8 ^{Aa}	24.0 ± 0.0 ^{Aa}	24.0 ± 1.4 ^{Aa}
3	21.0 ± 1.4 ^{Aa}	24.0 ± 2.8 ^{Aa}	25.5 ± 3.5 ^{Aa}
5	26.0 ± 2.8 ^{Aa}	20.5 ± 2.1 ^{Aa}	24.5 ± 0.7 ^{Aa}
a^*			
Ctrl		37.05 ± 0.92	
1	32.60 ± 1.56 ^{Aa}	32.50 ± 0.99 ^{Aa}	32.25 ± 1.06 ^{Aa}
3	33.85 ± 0.21 ^{ABa}	36.15 ± 0.21 ^{Bab}	36.20 ± 1.70 ^{Bab}
5	35.70 ± 2.12 ^{ABb}	30.50 ± 0.71 ^{Aa}	37.30 ± 1.27 ^{Bb}
b^*			
Ctrl		21.35 ± 0.49	
1	16.10 ± 0.42 ^{Aa}	18.25 ± 1.20 ^{Bb}	16.55 ± 0.21 ^{Aab}
3	17.55 ± 0.35 ^{ABa}	20.30 ± 1.70 ^{BCa}	20.50 ± 2.83 ^{Ba}
5	19.20 ± 1.41 ^{Bb}	14.60 ± 0.71 ^{Aa}	20.50 ± 0.71 ^{Bb}

Within each column, different uppercase letters (A, B, C**) indicate statistically significant differences between treatment duration and different lowercase letters (a, b, c) indicate statistically significant differences between applied amplitude ($p \leq 0.05$, Tukey post-hoc test).
***1, 3, 5 denote treatment time(min) and 30%, 40% and 50% denote treatment amplitude.

concentration ($p = 0.001$, $r = -0.3680$) but it remained relatively stable across different treatment conditions, confirming that ultrasound processing did not significantly affect the overall lightness of the samples. However, both a^* and b^* values showed noticeable variations depending on the applied amplitude and treatment duration. In several cases, increasing amplitude and/or treatment time resulted in a decrease in a^*, indicating a reduction in red colour intensity. Similar trends were observed for b^*, suggesting changes in the yellow colour component.

According to the calculated total colour differences (ΔE^*), all treated samples showed a value greater than 3.0 (except for 40% and 3 min), indicating that the colour changes are clearly perceptible to the human eye (Table S4, Supplementary materials). The greatest colour instability was observed at 40% amplitude for 5 min, reaching a maximum of 9.46. This suggests significant degradation of the visual profile or a high rate of pigment transformation at this specific concentration and time. Notably, the 50% amplitude group exhibited a decreasing trend in colour difference with increasing treatment duration, from $t = 1$ min (7.23) to $t = 5$ min (3.13), suggesting a potential stabilizing effect of higher concentrations on the food matrix over the course of treatment.

Colour changes in ultrasound-treated tomatoes reflect interactions between carotenoids, polyphenols, and matrix structural modifications. Colour parameters (L^*, a^*, b^*) were strongly associated with carotenoid content, confirming their dominant role in colour formation. Positive correlations between lycopene and a^* and b^*, as well as negative correlation with L^* indicate that red colour intensity is mainly driven by carotenoids. In contrast, the negative correlation between TPC and colour parameters suggests that increased phenolic extraction reduces carotenoid-derived colour intensity.

Although carotenoids are key contributors, colour reflects the combined effect of multiple pigments and matrix properties, including structural and optical changes induced by ultrasound. The strong positive correlation between a^* and b^* indicates coordinated variation of red and yellow components. These findings are consistent with previous studies Piazza et al. (2022), reported stable L^* values with decreased a^* and increased b^*. Similarly, Li et al. attributed L^* changes to ultrasound-induced shear forces improving light transmission (Li et al., 2025), while where Rojas and coauthors linked decreases in a^* and increases in b^* to cavitation effects and pigment release (Rojas et al., 2016). Overall, ultrasound influences colour through complex changes in chromaticity rather than individual pigment concentrations.

3.4. Antioxidant activity

The results of the antioxidant activity and concentration of antioxidants in tomato pulp samples are presented in Table 3 and Table S5, Supplementary materials. A statistically significant increase was observed with respect to treatment duration ($p < 0.0001$), amplitude ($p < 0.0001$), and their interaction ($p < 0.0001$) (Table S6, Supplementary materials). All ultrasonicated tomato pulp samples exhibited higher antioxidant activity than the control. The greatest reduction in EPR-DPPH signal intensity (78.63%) was observed in the sample treated for 1 min at 50% amplitude, while the lowest reduction among treated samples was found in the sample treated for 5 min at 30% amplitude (56.13%) (Table 3). Samples treated at 30% amplitude showed the lowest antioxidant activity, while those treated at 50% showed the highest. Increasing treatment duration enhanced antioxidant activity, with samples treated for 5 min exhibiting the highest antioxidant activity among all treated samples. Supporting this finding, the correlation analysis (Fig. S1, Supplementary materials) confirmed a positive correlation ($p = 0.017$, $r = 0.4193$) between antioxidant activity and applied amplitude. A weak negative correlation was observed between lycopene concentration and antioxidative activity ($p = 0.035$, $r = -0.3733$).

Table 3
Antioxidant activity (AA) of tomato pulp samples expressed as the percentage (%) reduction of EPR-DPPH signal intensity.

	Antioxidant activity (AA) % reduction of DPPH		
	30%	40%	50%
Ctrl	67.95 ± 0.01		
1 min	70.20 ± 0.01 ^{Ba}	69.37 ± 0.02 ^{Aa}	78.63 ± 0.01 ^{Cb}
3 min	67.85 ± 0.02 ^{Ba}	75.69 ± 0.03 ^{Bb}	74.59 ± 0.00 ^{Bb}
5 min	56.13 ± 0.02 ^{Aa}	72.36 ± 0.02 ^{ABc}	75.46 ± 0.01 ^{Bc}

Within each column, different uppercase letters (A, B, C**) indicate statistically significant differences between treatment duration and different lowercase letters (a, b, c) indicate statistically significant differences between applied amplitude ($p \leq 0.05$, Tukey post-hoc test).
***1, 3, 5 denote treatment time (min) and 30%, 40% and 50% denote treatment amplitude.

The results of antioxidant activity are in accordance with results of concentration of antioxidants presented in tomato pulp. The highest concentration was observed in sample treated for 1 min and 50% applied amplitude (327.4 μg/m), while the lowest (216.9 μg/mL) was treated for 5 min at 30% amplitude (Table S5, Supplementary materials). These trends reflect the downstream effects of ultrasound-induced structural modifications and changes in bioactive compound composition.

The results demonstrated a significant influence of processing parameters on the antioxidant activity of treated samples. Correlation analysis (Fig. S1, Supplementary materials) indicated a relationship between the applied amplitude and antioxidant properties ($p = 0.017$, $r = 0.4193$), where higher amplitudes combined with shorter treatment durations generally resulted in enhanced antioxidant activity. Although the treatment at 40% amplitude for 3 min yielded both high antioxidant activity and the maximum lycopene concentration, the overall correlation analysis revealed a moderate negative relationship between lycopene content and antioxidant activity ($p = 0.035$, $r = -0.3733$). The decrease in antioxidant activity observed under specific conditions (30% amplitude, 5 min) may be related to the complex response of bioactive compounds to prolonged ultrasound exposure. This apparent inconsistency suggests that antioxidant activity is not governed by a single compound class, but rather reflects a complex and multifactorial response. In this context, antioxidant activity arises from the combined and potentially synergistic interactions of various bioactive compounds, including polyphenols, carotenoids, and other redox-active molecules. Although total phenolic content generally increases with treatment intensity and duration, carotenoids (lycopene and beta-carotene) show a non-linear behaviour depending on processing conditions, which may contribute to variations in overall antioxidant response. At higher amplitudes, the increased release of phenolic compounds appears to support higher antioxidant activity despite concurrent changes in carotenoid levels, indicating a balance between extraction and structural modifications of bioactive compounds.

Lafarga and coauthors (Lafarga et al., 2019) attributed the increase in antioxidant activity of tomatoes to elevated polyphenolic content particularly highlighting kaempferol, myricetin, and rutin. In the research of Lu et al. (2020), the increase in antioxidant activity of tomatoes treated with ultrasound at a constant amplitude was positively correlated with the polyphenolic and ascorbic acid content of the samples, with shorter ultrasound durations generally enhancing both parameters. Nevertheless, the relationship of lycopene concentrations with the antioxidant activity highlights the contribution of carotenoids, supporting the concept that antioxidant behavior results from the synergistic action of multiple bioactive constituents rather than a single compound class. These findings reinforce the widely accepted view that antioxidant activity is a multifactorial response governed by interactions among phenolics, carotenoids, and other redox-active molecules. Although increases in bioactive compounds were accompanied by enhanced antioxidant responses, the absence of significant correlations indicates a complex interplay of mechanisms influencing antioxidant behavior.

3.5. Free radical determination

Previous ultrasound-assisted extraction studies in tomato systems have mainly focused on extraction yield and antioxidant activity, whereas direct monitoring of ultrasound-induced radical formation has been considerably less explored. In the present study, EPR spin trapping enabled observation of radical behaviour under different processing conditions, providing additional mechanistic insight into the balance between cavitation-induced oxidation and antioxidant protection. Fig. 3 shows a characteristic EPR signal of the DIPPMPO-OH spin adduct observed in this study, consisting of a multiplet described as two triplets split by a doublet, reflecting hyperfine interactions with nitrogen (^{14}N, $a_N = 2.7580$ mT), hydrogen (^{1}H, $a_H = 1.3424$ mT), and phosphorus (^{31}P, $a_P = 4.7334$ mT). The difference in EPR-DIPPMPO signal intensity between the two treatments (50%–5 min and 40%–1 min) is related to the concentration of the DIPPMPO spin adduct. An increase in the concentration of the DIPPMPO spin adduct leads to an increase in EPR signal intensity. It should be noted that EPR measurements in complex food matrices such as tomato pulp may be influenced by matrix effects, including the presence of endogenous antioxidants, pigments, and other redox-active compounds, which can affect spin trapping efficiency and signal intensity.

To better contextualise the results obtained in tomato pulp and to distinguish between radical formation inherent to the ultrasound process and the matrix-specific effects of tomato components, the same spin trapping experiments were conducted in Milli-Q water as in tomato pulp system. The comparison between water and tomato pulp samples indicates that ultrasound treatment generates a higher number of radicals in water than in the tomato pulp (Fig. 4). It should be noted that the untreated tomato pulp control exhibited a measurable DIPPMPO signal, indicating baseline radical formation originating from the Fenton system in the tomato matrix. Consequently, ultrasound effects were interpreted relative to this baseline, where the detected signal reflects the dynamic balance between radical generation and scavenging by endogenous antioxidant compounds. Across all treatments, an increase in ultrasound amplitude resulted in higher radical formation in both samples (tomato pulp and water). In contrast, prolonged treatment time (3 and 5 min) led to a decrease in radical levels, compared to the shorter treatment (1 min). This can be explained by the enhanced recombination of free radicals during prolonged treatments, leading to the formation of more stable, non-radical products that cannot be detected by EPR spectroscopy. In addition, the stability of DIPPMPO spin adducts may be affected by processing conditions and the chemical environment of the matrix, which should be considered when interpreting signal intensity as a relative rather than absolute measure of radical concentration.

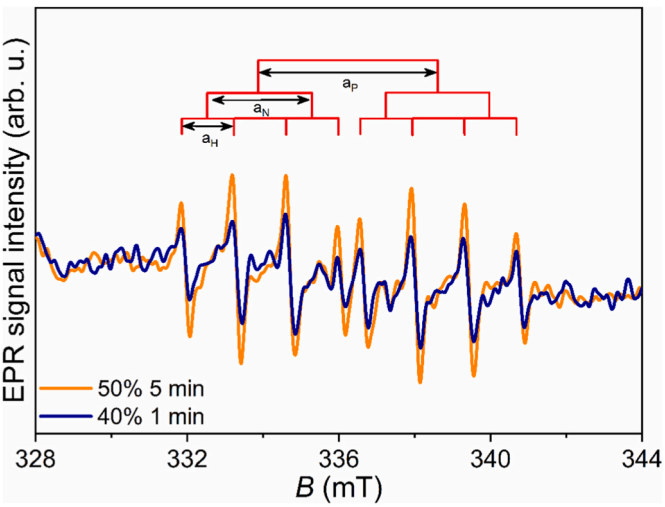

Fig. 3. Characteristic EPR signal of the DIPPMPO spin adduct in ultrasound-treated tomato pulp samples.

The observed increase in free radical formation with higher ultrasonic processing intensity can be mechanistically linked to acoustic cavitation (Smokrović et al., 2026). During cavitation, microscopic bubbles rapidly grow and collapse, generating localized hotspots characterized by extremely high temperatures and pressures. These extreme conditions promote the thermal dissociation of water and other molecules, producing reactive species such as hydroxyl and hydrogen radicals (Smokrović et al., 2026). The formation of these radicals is consistent with the primary sonochemical reactions occurring during acoustic cavitation, where water molecules undergo thermal dissociation inside collapsing microbubbles:

$$H_2O \rightarrow H\bullet + \bullet OH$$

Subsequently, radical recombination reactions such as:

$$\bullet OH + \bullet OH \rightarrow H_2O_2$$

further contribute to the formation of secondary, more stable species.

These processes confirm that the observed EPR signals originate from cavitation-driven water sonolysis and subsequent radical transformation pathways (Trinh et al., 2025; Wu et al., 2025).

This sonochemical radical generation has been confirmed by spin trapping and EPR studies and is known to be influenced by processing parameters such as acoustic power, frequency, and temperature (Akti & Yildiz, 2025; Pétrier, 2015). With increasing amplitude and longer treatment duration, cavitation becomes more intense, promoting the formation of free radicals and enhancing oxidative reactions in the food matrix (Ashokkumar, 2015; Chemat et al., 2017). Under these conditions, ultrasonic processing may simultaneously promote free radical formation while enhancing the release or availability of antioxidant compounds from the food matrix, indicating a complex interaction between oxidation processes and antioxidant activity.

The lower radical levels observed in tomato pulp samples are explained by the presence of bioactive compounds, particularly antioxidants, which scavenge and neutralize the generated radicals. In addition, the concentration of antioxidants (Table S5, Supplementary materials) increased with extended treatment time. As a result, the presence of these antioxidants contributes to the scavenging of newly formed free radicals, further reducing their detectable concentration. The reduction of the EPR-DIPPMPO signal to a minimum of 72.5 at the 3 min at 40% treatment (corresponding to an inhibition of 34.1%) marks the point at which cavitation released the optimal amount of polyphenols. These polyphenols effectively intercepted the ·OH radicals, preventing their detection by the spin-trap agent. The decrease in inhibition at 5 min and 50% amplitude indicates that the extreme sonochemical production of radicals exceeded the antioxidant activity of the tomato pulp, which correlates with the previously observed degradation of lycopene. In agreement with this concept, the present results indicate a clear relationship between DIPPMPO spin adducts and antioxidant components, particularly lycopene. A decrease in lycopene content was accompanied by an increase in DIPPMPO spin adduct levels, whereas higher lycopene concentrations corresponded to lower radical signals, highlighting the important role of lycopene as a scavenger of ultrasound-induced free radicals. On other hand, the correlation analysis (Fig. S1, Supplementary materials) revealed a significant positive relationship between beta-carotene concentration and DIPPMPO spin adducts ($p = 0.000$, $r = 0.5818$), while no significant association was observed between beta-carotene and the applied processing parameters. This indicates that the observed relationship is not directly influenced by ultrasound conditions, but rather reflects underlying interactions within the sample matrix. The positive correlation may be attributed to the limited ability of beta-carotene to effectively scavenge the radical species detected by EPR spectroscopy, particularly due to its lipophilic nature and reduced reactivity in aqueous systems. Consequently, the

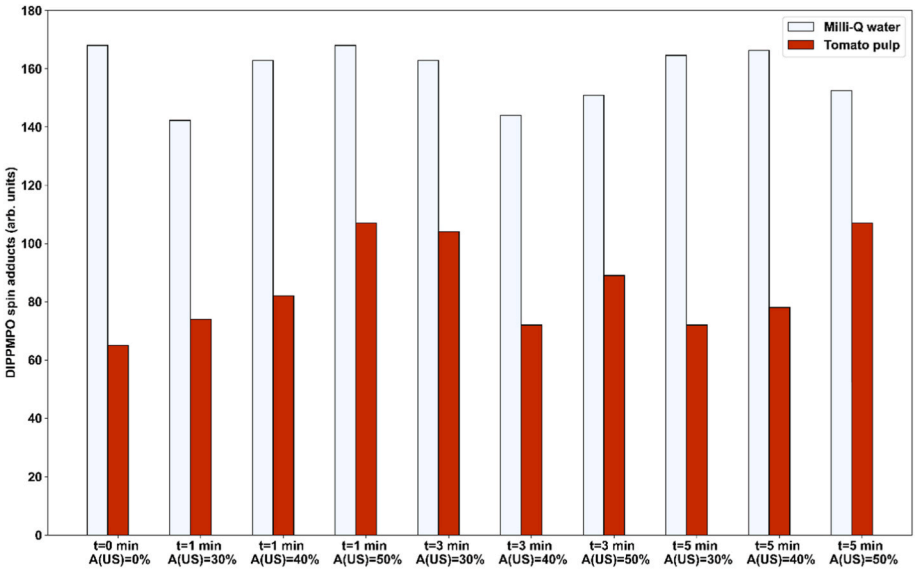

Fig. 4. DIPPMPO spin adducts in ultrasonicated tomato pulp (orange) and Milli-Q water (blue), determined by EPR spin trapping and expressed as integrated signal intensities.

presence of higher beta-carotene concentrations does not necessarily correspond to a decrease in detectable radical species. Therefore, the observed association likely reflects the complex and multifactorial nature of the system, rather than a direct causal relationship between beta-carotene and radical formation.

To the best of our knowledge, no studies have directly monitored free radical formation in ultrasonically processed tomato pulp samples. However, similar observations are obtained in the research of Smokrović et al. (Smokrović et al., 2026), who reported that ultrasonic treatment in tomato juice simultaneously enhances radical generation through cavitation while also promoting the release of intracellular bioactive compounds. Their results suggest that the net radical signal depends on the balance between sonochemically induced radical formation and the availability of antioxidant molecules capable of scavenging these species. This dynamic interplay is particularly important in complex food matrices, where ultrasound can both induce oxidative processes and increase the extractability of antioxidant compounds. Furthermore, similar observations have been reported in other food matrices, such as cherry juice, where increased radical production was observed at higher ultrasound intensities (Akti & Yildiz, 2025). This effect was attributed to cavitation-induced localized pyrolysis and sonochemical reactions that promote radical formation. Comparable trends have also been reported in liquid food systems, including red and model wine, where ultrasound power and treatment time significantly affected radical generation (Zhang et al., 2015). Overall, these findings indicate that radical formation increases with ultrasonic intensity, emphasizing the strong influence of processing parameters on sonochemically induced oxidation.

3.6. Correlation and principal component analysis (PCA)

In this study, multivariate statistical analysis (PCA) and analysis of variance (ANOVA) were applied, and the Pearson correlation matrix was calculated (Fig. S1, Supplementary materials) to determine the influence of process time and amplitude on the key quality parameter. A summary of significant correlations is presented in Table S7, Supplementary materials. On a PCA biplot, the angle between the vectors representing the variables directly visualizes the Pearson correlation coefficient, r; that is, angle $\approx$ arccos (r). Principal component analysis (PCA) showed that the first two components explain a total of 63.72% of the system variability, with PC1 contributing 41.44% and PC2 22.28% (Fig. 5). This high percentage of explained variance confirms a strong correlation between

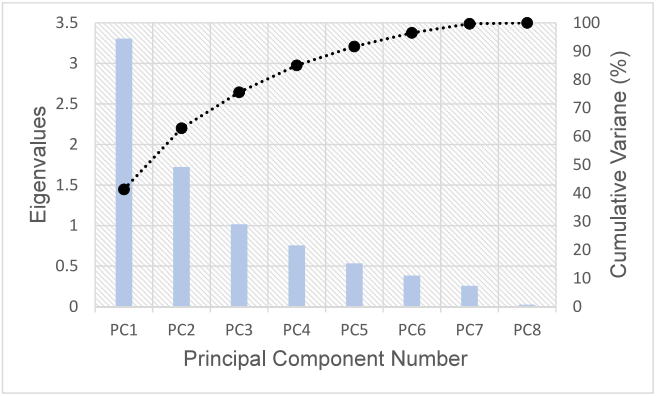

Fig. 5. PCA Scree and cumulative variance plots.

the applied ultrasound treatment and changes in the physicochemical profile of the tomato pulp samples. PCA loadings plot is presented at

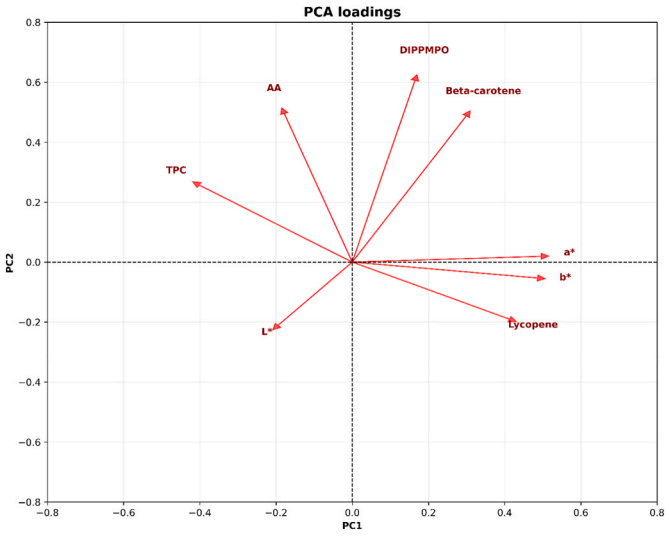

Fig. 6. Principal component analysis (PCA) loadings plot.

Fig. 6.

PC1 is primarily defined by colour (a^*, b^*) and lycopene content, in contrast to total phenolics (TPC). The strongest positive influences are a^*, b^*, and lycopene, in descending order, with a negative influence from TPC. The most dominant variable in PC2 is the number of DIPPMPO spin adducts, followed by the strong positive influence of antioxidative activity and beta-carotene. Interestingly, L^* (−0.1947 and −0.2109) has relatively small weights on both components, meaning that "brightness" (L^*) contributes least to pattern discrimination in this study, as shown by the equations of the principal components:

$$\begin{aligned}\mathrm{PC1} = &-0.401888\ \mathrm{TPC} + 0.41254\ \mathrm{Lycopene} + 0.299045\ \mathrm{Betacarotene}\\ &- 0.178341\ \mathrm{AA} - 0.1947\ \mathrm{L^*} + 0.495682\ \mathrm{a^*} + 0.486232\ \mathrm{b^*}\\ &+ 0.164418\ \mathrm{DIPPMPO}\end{aligned} \tag{5}$$

$$\begin{aligned}\mathrm{PC2} = &\ 0.257233\ \mathrm{TPC} - 0.19029\ \mathrm{Lycopene} + 0.487314\ \mathrm{Betacarotene}\\ &+ 0.495447\ \mathrm{AA} - 0.210927\ \mathrm{L^*} + 0.0195172\ \mathrm{a^*} - 0.0531306\ \mathrm{b^*}\\ &+ 0.605792\ \mathrm{DIPPMPO}\end{aligned} \tag{6}$$

where the values of the variables in the equation are z-standardized by subtracting their means and dividing by their standard deviations.

3.6.1. Geometrical vector relationships

A strong positive correlation is evident from the smallest angle between a^* and b^* (8.49°), indicating that the two variables behave almost identically. Furthermore, beta-carotene and DIPPMPO spin adducts (16.35°), and lycopene and b^* (18.53°) have very small angles, confirming strong positive correlations. The strongest negative correlations are observed for TPC and lycopene (172.14°), and beta-carotene and L (168.83°), which are on almost opposite sides. In contrast, TPC and beta-carotene are almost at right angles (88.91°), indicating that there is almost no linear relationship between them.

The angle between lycopene and a^* is very small (27°), confirming that the increase in lycopene concentration is almost perfectly accompanied by a more intense red colour. The smaller angle with b^* (18.53°) compared to a^* suggests that lycopene is not purely red but contributes to the overall intensity of the warm colour in the red-yellow spectrum. The angle (about 70°) between lycopene and beta-carotene indicates that lycopene levels are not related to beta-carotene levels.

3.6.2. Linking PCA projections to processing conditions (time and amplitude)

The spatial separation of sample coordinates on the PC1 vs. PC2 score plot establishes a trajectory that directly reflects the intensity of the processing conditions:

- Control and low-intensity region (0 min and 1 min/30%): These samples were located in the positive region of PC1, closely associated with high loadings of lycopene and colour parameters a^* and b^*. The ultrasound energy applied under these conditions is insufficient to cause significant degradation of bioactive compounds. This indicates that minimal ultrasound exposure preserves the native carotenoid-rich structure of tomato pulp, maintaining higher colour intensity and overall pigment integrity.
- Optimal extraction region (1–3 min/40% and 5 min/30%): With increasing processing time and moderate ultrasound intensity, samples shifted towards the positive PC2 and negative PC1 region. This region can therefore be interpreted as the optimal extraction zone, where acoustic cavitation and microstreaming enhance cell wall disruption and facilitate the release of bound hydrophilic phenolic compounds. This is consistent with the observed increase in TPC and antioxidant activity, indicating improved functional quality without excessive pigment degradation.
- Over-processing and degradation region (3–5 min/50% and 5 min/40%): Severe processing conditions were positioned in the extreme negative region of PC1, opposite the pigment-related vectors (lycopene, a^*, and b^*). This position highlights a critical technological trade-off. Prolonged processing time combined with high ultrasound amplitude (50%) generates intense acoustic cavitation, leading to localised micro-heating and oxidative reactions. These effects contribute to the structural breakdown of sensitive bioactive compounds, particularly lycopene, accompanied by a reduction in colour intensity (a^* and b^* values).

3.6.3. Biochemical mechanisms and radical scavenging dynamics

The strong negative correlation between lycopene and TPC along the PC1 axis (172.14°) further supports this trade-off: processing conditions that maximise the liberation of polyphenols through cell disruption simultaneously compromise the retention of red and yellow pigments. Conversely, the very small angle between TPC and AA (only 21.5°) indicates a strong positive correlation. The vectors point in almost the same direction, confirming that total phenolics (TPC) are the primary contributors to antioxidant activity in our samples. Any increase in phenolics caused by ultrasound is directly accompanied by an increase in antioxidant activity. Furthermore, the negative correlation between TPC and DIPPMPO spin adducts (representing residual ·OH radicals) suggests that phenolic compounds are the most effective in scavenging these radicals. In contrast, beta-carotene showed a positive relationship with the level of the DIPPMPO signal. This result indicates that, in our samples, carotenoids were not the primary factors in neutralising ·OH radicals, despite their known antioxidant potential. Additionally, the independence of lycopene and colour parameters (a^* and b^*) in relation to DIPPMPO spin adducts confirms that the visual colour intensity of the sample is not a reliable indicator of its biochemical effectiveness against this specific type of free radical.

In conclusion, the PCA results suggest that optimising ultrasound processing requires precise, simultaneous regulation of both amplitude and time. Intermediate settings are key to achieving maximum phenolic release and biological activity against ·OH radicals while avoiding irreversible thermal and oxidative loss of visual pigment quality.

4. Conclusion

This study demonstrated that ultrasound-assisted processing significantly affects the extraction of bioactive compounds, antioxidant activity, colour parameters, and free radical formation in tomato pulp. Total polyphenolic content and antioxidant activity increased with treatment intensity (TPC up to 0.380 mg/mL and antioxidant activity up to 78.63%), whereas lycopene decreased from 2.933 to 1.773 μg/mL under more severe conditions and beta-carotene remained largely unaffected. EPR spin-trapping analysis showed that free radical formation depended on ultrasound amplitude and treatment duration, emphasizing the importance of processing conditions in modulating oxidative reactions.

The present findings provide clear evidence of the dual nature of ultrasound processing, where enhanced extraction of bioactive compounds occurs simultaneously with sonochemically induced free radical formation driven by acoustic cavitation. The results suggest that antioxidant compounds, particularly polyphenols, may modulate ultrasound-induced free radical formation, highlighting the potential of ultrasound as a tunable non-thermal technology for controlling oxidative processes and improving the functional quality of plant-based foods. Although these findings highlight the potential of ultrasound for modulating extraction and oxidation–reduction processes in food systems, the present study was conducted at laboratory scale, and further research is required to evaluate scalability and process uniformity under industrial conditions.

CRediT authorship contribution statement

Franka Markić: Writing – review & editing, Writing – original draft, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Senada Muratović:** Writing – review & editing, Writing – original draft, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Sanda Pleslić:** Writing – review & editing, Visualization, Formal analysis, Data curation. **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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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.lwt.2026.119628.

Data availability

Data will be made available on request.

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