

Optimizing pulsed electric field and high-power ultrasound treatments to preserve anthocyanin stability and physicochemical quality in stored strawberry juice

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Abstract

This study presents a novel approach to preserving the quality of strawberry juice through the innovative combination (hurdle) of pulsed electric field (PEF) and high-power ultrasound (HPU) treatments. The objective was to evaluate the impact of various PEF (30 kV/cm, 100 Hz, 1.5-4.5 min) and HPU (25% amplitude, 50% pulse, 2.5-7.5 min) treatments on anthocyanin stability, color, and physicochemical properties (conductivity, browning index, dissolved oxygen, and hydroxymethylfurfural content) during 7-day storage at 4°C. Our findings reveal that storage significantly influenced anthocyanin content, physicochemical properties, and color. The combined PEF (3 min) and HPU (2.5-7 min) treatments markedly enhanced anthocyanin stability compared to the control samples. Importantly, this combined non-thermal treatment approach significantly affected all studied parameters except for hydroxymethylfurfural content. Optimal anthocyanin content was achieved with a PEF treatment of 2.19 min and an HPU treatment of 7.48 min over 7 days of storage, while minimal color changes were observed with PEF (3.14 min) + HPU (7.50 min). This study demonstrates the potential of combining PEF and HPU as a feasible and effective strategy for processing functional strawberry juices, ensuring anthocyanin stability and desirable physicochemical properties.

Keywords: browning index; conductivity; high-power ultrasound; hydroxymethylfurfural; non-thermal hurdle technologies; optimization; oxygen content; pulsed electric field

Introduction

As with different types of berries (Zhang *et al.*, 2023), the strawberry (*Fragaria × ananassa* Duch.) is also a popular fruit due to its high nutritional (e.g. polyphenolic) and commercial values (Warner *et al.*, 2021). Many studies on the antioxidant, anti-inflammatory, antihypertensive, and

antiproliferative properties of strawberries have shown that strawberry products (e.g., juices) have significant bioactive potential because of the high content of polyphenols (Cosme *et al.*, 2022). The attractive red color of strawberries is attributed to anthocyanins, among their most critical polyphenolic compounds. The anthocyanin content in strawberries depends on numerous factors,

such as cultivar, ripeness, growing, storage conditions (Bebek Markovinović *et al.*, 2023; Crecente-Campo *et al.*, 2012). Due to their high thermolability, they rapidly degrade during processing and thus lose their color and bioactive properties. Numerous factors have been identified that affect strawberry quality (Bebek Markovinović *et al.*, 2024a), hence special attention must be dedicated to its protection during processing to obtain a high-quality final product (Enaru *et al.*, 2021).

The most sensitive step in the production of strawberry juice, which significantly impacts the final product's quality, is pasteurization, as heat treatments usually lead to the deterioration of the food's nutritional, sensory, and phytochemical properties. Heat treatment of juice can lead to browning, the formation of hydroxymethylfurfural (HMF), color changes, and the loss of bioactive compounds (Galanakis, 2021). However, major changes in juice quality can also occur during storage (Chen *et al.*, 2020). The simultaneous degradation of anthocyanins, the fading of the red color, and the formation of brown pigments due to enzymatic and/or non-enzymatic reactions lead to undesirable color changes. The brown color of pasteurized strawberry juice during storage is mostly caused by non-enzymatic processes such as Maillard reactions and acid-catalyzed sugar degradation, as heat treatment inhibits the activity of quality-degrading enzymes (Buvé *et al.*, 2018).

Consumers today prefer affordable fruit juices with qualities as close to fresh fruits (e.g., fresh or cold-pressed juices). Such products should have excellent nutritional and functional qualities and a long shelf life (Martins *et al.*, 2019; Yildiz *et al.*, 2021) while replacing synthetic additives with natural alternatives from plants (Gladikostić *et al.*, 2023). Fruit production technology is steadily being transformed with non-thermal alternatives, not only for pasteurization but also for other operations, for example, homogenization, such as emulsification of pectins (Gharibzahedi *et al.*, 2019).

Recently, the application of hurdle technology, i.e., the simultaneous application of several technologies in lower intensity treatments than if each were applied alone, has attracted much attention. This concept offers numerous advantages in terms of the final quality of the product during storage and, at the same time, good preservation of the nutritional, biological, and sensory properties of the product, which is why it has great potential for sustainable industrial application (Dixit *et al.*, 2018; Djekić *et al.*, 2023).

The combined use of non-thermal technologies such as pulsed electric field (PEF), high hydrostatic pressure (HHP), high-power ultrasound (HPU), and cold plasma (CP) in juice processing is being extensively investigated (Putnik *et al.*, 2020). However, the combined use

of PEF and HPU in processing strawberry juice, a very demanding fruit matrix, has not yet been sufficiently researched. PEF and HPU were chosen for this study due to their complementary mechanisms and proven potential to enhance the stability of bioactive compounds. PEF uses short bursts of high voltage to disrupt cell membranes through electroporation, facilitating the extraction of intracellular compounds (Graybill & Davalos, 2020). HPU, on the other hand, generates cavitation bubbles that collapse and produce intense shear forces, improving mass transfer and extraction efficiency (Barba *et al.*, 2020). Evidence suggests that combining these two technologies is expected to synergistically enhance anthocyanin stability, reduce browning, inactivate microorganisms and enzymes, and maintain desirable physicochemical properties in strawberry juice (Guerrero-Beltrán & Welti-Chanes, 2016).

Therefore, in this study, the combined effects of PEF (30 kV/cm, 100 Hz) for 1.5, 3.0, and 4.5 min and HPU (amplitude 25%, pulse 50%) for 2.5, 5.0, and 7.5 min on color changes, physicochemical properties (oxygen content, conductivity, browning index, and HMF content), and anthocyanins in strawberry juice during 7 days of storage at 4°C were investigated using chemometrics. The aim was to investigate the quality of juices treated as a “fresh-like” product that would interest consumers. Finally, optimization was performed to determine the best processing parameters for combined PEF and HPU treatments with the highest anthocyanin yield, the least color change, and browning, in addition to the lowest content of HMF.

Materials and Methods

Production of strawberry juices

The strawberries (*Fragaria × ananassa* Duch.) cv. ‘Albion’ used for juice preparation were purchased from Jagodar HB d.o.o. (Donja Lomnica, Croatia). After transportation to the laboratory, the fruits were washed, stalks were removed, then dried, and stored at -18°C. The pH of the raw material was 3.23, the soluble solids content (SSC) was 9.85%, and the total acidity was 0.89%. The day before the experiment, the fruits were thawed overnight and then processed into juice using a Kuvings B6000 (VerVita d.o.o., Zagreb, Croatia) cold press juicer (240 W, 60 rpm) with a filter diameter of 0.2 mm.

Pulsed electric field (PEF) and high-power ultrasound (HPU) treatment of strawberry juices

Considering the optimized process parameters for the PEF and HPU technologies determined in previous studies (Bebek Markovinović *et al.*, 2022a; Bebek

Markovinović *et al.*, 2022c), the experimental design for the combined treatment of PEF followed by HPU was established (Table 1).

PEF treatments were performed using an HVG60/1 PEF device (Impel d.o.o., Zagreb, Croatia), as previously reported (Bebek Markovinović *et al.*, 2022c). A 200 mL batch treatment chamber was equipped with two parallel stainless-steel electrodes, each with a diameter of 68 mm and a distance of 25 mm. The electric field strength was set to 30 kV/cm, with a pulse frequency of 100 Hz and a constant pulse width of 1 μ s.

Immediately after the PEF treatment, the samples were subjected to HPU treatment. The Hielscher UP400St High Power Sonicator, 400 W, 24 Hz, with DN22 (surface area 546 mm² and diameter 2.2 cm) titanium sonotrode (Hielscher Ultrasonics GmbH, Germany) was used as previously described (Bebek Markovinović *et al.*, 2022a). The juice samples were treated with an amplitude of 25% and a pulse of 50%. A 200 mL of the sample was treated in a glass beaker immersed in a cold-water bath with ice to minimize the impact of temperature during the treatment. During the treatment, the sonotrode was positioned at the center of the glass beaker and immersed to a depth of 2.5 cm in the sample. The temperature of the treated juices was monitored before and after PEF and HPU treatment using the PCE-777 thermometer (PCE-Instruments, UK). The average temperature of the samples before and after all treatments did not exceed 19.55°C. Therefore, the

effect of temperature on all observed dependent variables was not considered in this study. After the treatments, one batch of juices was analyzed, while the other batch was stored at 4°C for 7 days and then analyzed. All juices were stored in sterile, securely sealed glass bottles (250 mL), filled to the top, in a dark place.

Determination of electrical conductivity

The electrical conductivity of the samples was measured in duplicate using a HI-2030-Edge conductivity meter (Hanna Instruments, USA). The results were expressed in μ S/cm of the juice sample.

Determination of dissolved oxygen

The dissolved oxygen of the samples was measured in duplicate using a SevenGo Duo Pro SG68-FK2 device (Mettler-Toledo GmbH, Greifensee, Switzerland). The results were expressed in mg/L of the juice sample.

Instrumental color measurements

Color measurements for all trials were conducted using a Konica Minolta Spectrophotometer (CM-700d, Konica Minolta, Japan) equipped with a D65 light source, 10° standard observer, and a target mask with an 8 mm aperture, plate, and open cone. A 20 mL of sample was pipetted into an optical glass cell CR-A504 (Konica Minolta, Japan), placed onto a plate of the target mask, and covered with a zero calibration box CM-A182 (Konica Minolta, Japan) to avoid the influence of ambient light. Colorimetric variables (L^* , a^* , b^*) were measured and color change (ΔE_{ab}^*), chroma (C^*), and hue (H^*) were calculated as (Bursać Kovačević *et al.*, 2016):

$$\Delta E_{ab}^* = \sqrt{\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2}} \quad (1)$$

$$C^* = \sqrt{a^{*2} + b^{*2}} \quad (2)$$

$$H^* = \tan^{-1} \left(\frac{b^*}{a^*} \right) \quad (3)$$

where all ΔL^{*2} , Δa^{*2} and Δb^{*2} were calculated in reference to the untreated samples. All measurements were done in triplicate.

Determination of browning index (BI) and content of hydroxymethylfurfural (HMF)

The BI was determined according to the method described in the literature (Cohen *et al.*, 1998). Briefly,

Table 1. Experimental design.

Sample ID	PEF exposure (min)	HPU exposure (min)	Storage (days)
1	1.5	2.5	0
2	1.5	5	0
3	1.5	7.5	0
4	1.5	2.5	7
5	1.5	5	7
6	1.5	7.5	7
7	3	2.5	0
8	3	5	0
9	3	7.5	0
10	3	2.5	7
11	3	5	7
12	3	7.5	7
13	4.5	2.5	0
14	4.5	5	0
15	4.5	7.5	0
16	4.5	2.5	7
17	4.5	5	7
18	4.5	7.5	7

5 mL of 96% ethanol was added to 5 g of strawberry juice, and the mixture was centrifuged at 6500 rpm for 10 min. The supernatant was separated from the precipitate, and one part was used to determine the BI, while the other part was used to determine HMF by spectrophotometry.

To determine the BI, the absorbance was measured at 420 nm with distilled water as blank. The BI was calculated using the following formula:

$$BI = A_{420\text{ nm}} - DF \quad (4)$$

where DF is the dilution factor, and $A_{420\text{ nm}}$ is the measured absorbance at 420 nm.

The HMF content was determined using a method described in the literature (Cohen *et al.*, 1998). Briefly, 2 mL of the supernatant (adequately diluted with distilled water), 2 mL of 12% trichloroacetic acid, and 2 mL of 0.025 M thiobarbituric acid were mixed in a test tube and incubated at 40°C for 50 min. The contents of the test tubes were then cooled for a few minutes with a stream of cold tap water, and the absorbance was measured at 443 nm. A blank sample was prepared similarly, but distilled water was used instead of the supernatant. All measurements were performed in duplicate. The results of the HMF content were calculated using a calibration curve prepared from standard solutions of different HMF concentrations (2.5, 5, 7.5, 10, 15 and 20 mg/L). A brief protocol for the determination of HMF involves mixing 2 mL of the standard solution with 2 mL of 12% trichloroacetic acid and 2 mL of 0.025 M thiobarbituric acid. The mixture is vortexed briefly and incubated at 40 °C for 50 minutes in a thermostatic bath. After incubation, the sample is rapidly cooled using cold tap water, and the absorbance is measured at 443 nm. A blank is prepared similarly, substituting distilled water for the standard solution. Results are expressed as mg HMF/L in the sample.

Determination of monomeric anthocyanins

The determination of monomeric anthocyanins in strawberry juice extracts was carried out using an ultrasound-assisted extraction method. A 5 g sample of strawberry juice was mixed with 20 mL of 1% formic acid in 80% methanol (v/v). The mixture was treated in an ultrasonic bath (DT 514 H Sonorex Digitec, 13.5 L, 860 W, 40 kHz, Bandelin Electronic, Berlin, Germany) at 50 °C for 15 min. Following the extraction, the solution was filtered, and the supernatant was transferred to a 25 mL volumetric flask, and diluted with the extraction solvent. The extracts were stored at -18 °C under an inert gas atmosphere until analysis (Bebek Markovinović *et al.*, 2024b). The method described in the literature was used to determine monomeric anthocyanins (AOAC, 1990).

In brief, 1 mL of the extract and 4 mL of buffer pH 1 (potassium chloride buffer, 0.025 M) were pipetted into a test tube, while 1 mL of the same extract was mixed with 4 mL of buffer pH 4.5 (sodium acetate buffer, 0.4 M) in a second test tube. After 20 min, absorbance was measured at 520 nm and 700 nm on an LLG-uniSPEC 2 spectrophotometer (Lab Logistics Group GmbH, Meckenheim, Germany), using deionized water as a blank. All measurements were performed in parallel, and the concentration was expressed as pelargonidin-3-glucoside equivalent (Pg-3-G) (mg/100 mL) according to the equation:

$$\frac{A \times MW \times DF \times 10^3}{\epsilon \times l} \quad (4)$$

where are:

$A = (A_{520\text{nm}} - A_{700\text{nm}})_{\text{pH}=1.0} - (A_{520\text{nm}} - A_{700\text{nm}})_{\text{pH}=4.5}$
 MW - molecular weight for pelargonidin-3-glucoside
 DF - dilution factor
 ϵ = molar absorption extinction coefficient
 l = thickness of the cuvette.

Statistical analysis

Experiments were designed as full factorial randomized designs ($n=40$). Dependent variables were the content of monomeric anthocyanins, electrical conductivity, browning index, content of hydroxymethylfurfural, CIELab variables, and dissolved oxygen in juice. Independent variables included: exposure to PEF (1.5, 3.0, 4.5 min), exposure to HPU (2.5, 5.0, 7.5 min), and length of storage (0 and 7 days). Descriptive statistics were used to assess the basic information about the experimental dataset. Differences in treatments (continuous variables) were tested by multivariate analysis of variance (MANOVA). Exploratory hierarchical Ward's cluster analysis was used to measure standardized similarities in samples. Nonparametric analysis employed the Kruskal–Wallis test. The significance level for rejection of a null hypothesis in all tests were $\alpha \leq 0.05$. Linear regression was employed to build and compare mathematical models. The significance level for all tests was $\alpha \leq 0.05$, all variance inflation factors were ≤ 5 . Only statistically significant predictors were retained in the models ($p \leq 0.05$). Analyses were performed with IBM SPSS Statistics (v.24), and the experimental design was performed by Statgraphics Centurion® (StatPoint Technologies, Inc, VA, USA).

Results

Chemometric evaluation of the influence of processing on the physicochemical properties, anthocyanins, and color stability of strawberry juices during storage

An exploratory hierarchical Ward's cluster analysis was performed to determine which samples in the data set had

similar standardized investigated properties (Figure 1). Samples treated with PEF for 3.0-4.5 min and HPU 2.5-7.5 min on day 0 of storage were most similar to controls (untreated samples) on day 0. Interestingly, after 7 days of storage, the control samples exhibited similar properties to those treated with PEF for 1.5-3.0 min and those treated with PEF for 1.5-3.0 min and HPU for 2.5-5.0 min. Since the stability of bioactive compounds, especially anthocyanins, depends on the duration of treatment, prolonged treatment is not conducive to the preservation of these compounds (Maza *et al.*, 2020). When comparing the results of the control samples with the most similarly treated samples, it was found that the intensification treatments had a similar effect on storage (0 vs. 7 days) in terms of the duration of the extension treatment. This was observed in the additional analysis below.

Table 2 shows the numerical values of the significance of the Kruskal–Wallis test between the treated and the control samples. The treated samples differed from the control samples with respect to conductivity and the color parameters a^* , b^* , C^* , H^* , and ΔE^*_{ab} . The median values of these results are shown graphically in Figure 2.

In general, the control samples differed from the treated samples, as they had lower median values for conductivity and all higher values for the CIELab parameters, except ΔE^*_{ab} , which was lower (Figure 2). Some literature reports suggest that the treated samples have a higher conductivity than the untreated ones, considering that the electrical conductivity of the sample increases with the degree of damage to its tissue due to the disintegration of the cell membrane by the phenomena of electroporation and cavitation during PEF and HPU treatment (Lebovka *et al.*, 2002). However, other findings have shown that the electric field influences the electrical conductivity. Therefore, the results obtained are in agreement with those of (Cserhalmi *et al.*, 2006) who found significantly higher conductivity values in treated grapefruit and orange juices than untreated ones. In addition, the control samples had higher median values of the color parameters a^* , b^* , C^* , and H^* than the treated samples, suggesting that the combination of PEF and HPU technologies significantly influenced juice color. However, this influence did not result in the degradation of anthocyanins, the formation of HMF, or an increase in BI, as no statistically significant differences were found

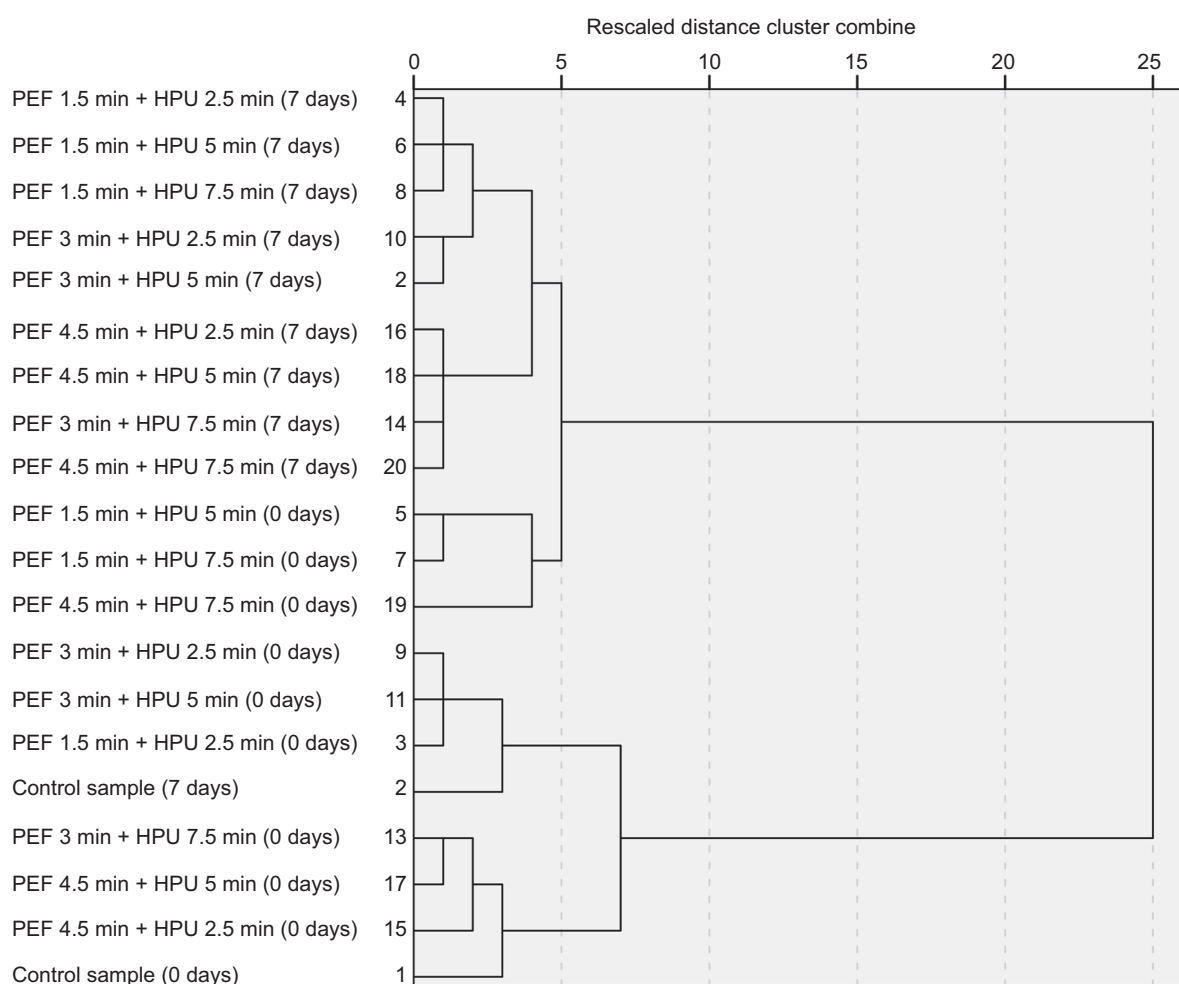


Figure 1. Results of the hierarchal cluster analysis of the averaged and standardized samples.

Table 2. Kruskal–Wallis test statistics for samples treated by combined technology vs. untreated samples.

	ANT	COND	BI	HMF	L*	a*	b*	C*	H*	ΔE^*_{ab}	O ₂
Chi-Square	3.09	7.83	0.46	2.94	3.59	7.81	7.32	7.81	4.30	7.81	0.00
df	1	1	1	1	1	1	1	1	1	1	1
Sig.	0.08	≤ 0.01*	0.50	0.09	0.06	≤ 0.01*	≤ 0.01*	≤ 0.01*	0.04*	≤ 0.01*	0.97

The Kruskal–Wallis test is significant at $p \leq 0.05$. ANT-Monomeric Anthocyanins (mg/100 g); COND-Conductivity ($\mu\text{S}/\text{cm}$); BI-Browning index; HMF-Hydroxymethylfurfural (mg/L); L-lightness; a*, b*-CIELab coordinates; C*-chroma; H*-hue; ΔE^*_{ab} -color change; O₂-dissolved oxygen in juice (mg/L).

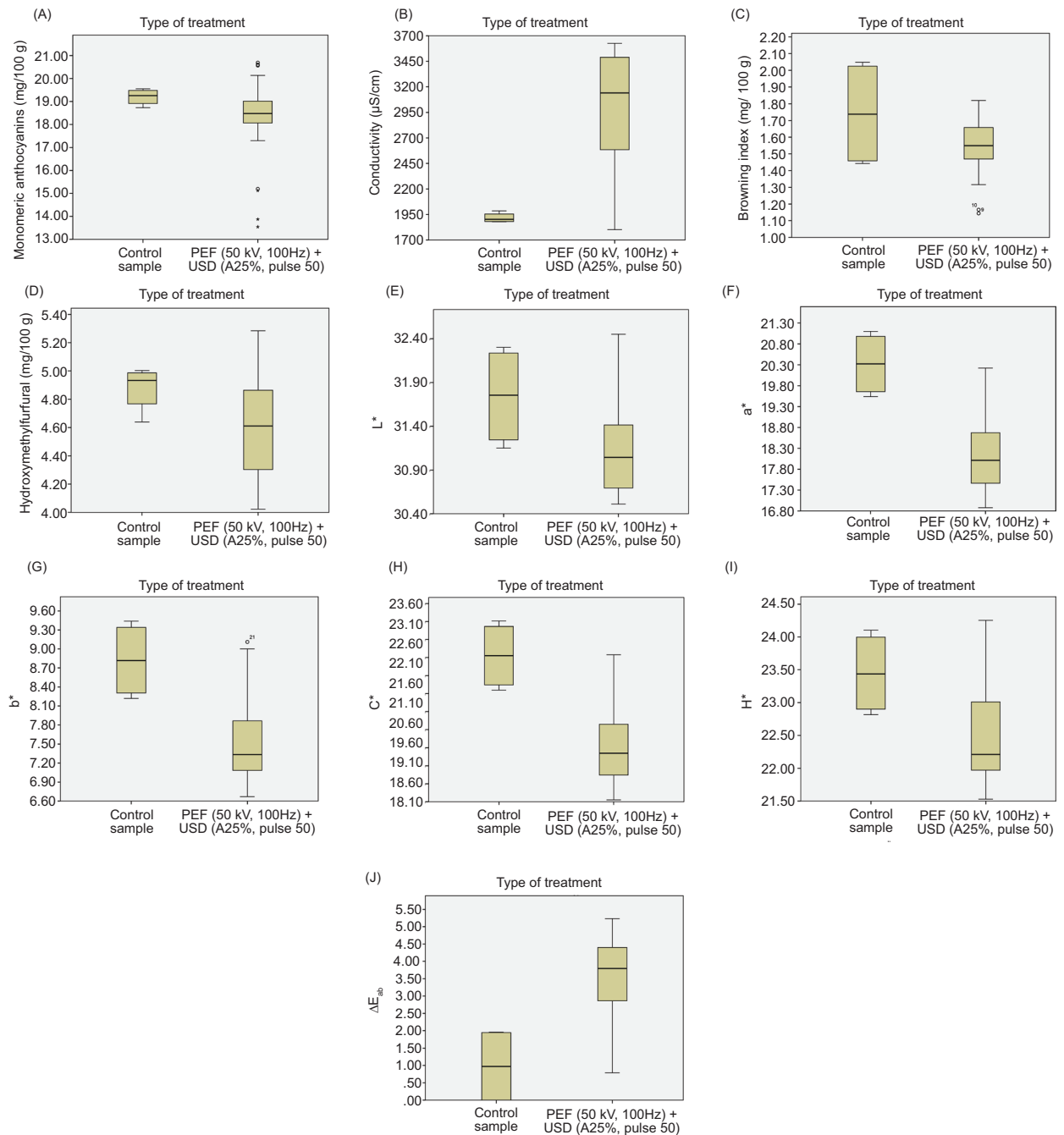


Figure 2. Median values of monomeric anthocyanins, physicochemical and color parameters in untreated vs. treated juice samples.

between the control and treated samples regarding these parameters. Other variables did not differ significantly from the control samples.

The influence of pulsed electric field (PEF) in combination with high-power ultrasound (HPU) on the physicochemical properties of strawberry juices during storage

The results of the effects of storage, PEF, and HPU treatments on the conductivity, dissolved oxygen, browning index, and HMF content in strawberry juices are shown

in Table 3. Storage significantly affected all parameters studied, with an increase in conductivity, browning index, and HMF content observed after 7 days of storage, while dissolved oxygen was lower at 0 days of storage. Increased conductivity in treated juices during storage was previously explained by increased extraction of intracellular contents during processing, resulting in mechanical damage to cell membranes (Lebovka *et al.*, 2001). The browning index and HMF content increased significantly at the end of storage compared to the initial values. This was expected due to the formation of HMF in the stored fruit juices, which could be associated with various degradation reactions, such as non-enzymatic

Table 3. Influence of storage, PEF, and HPU treatments on the conductivity, dissolved oxygen, browning index, and HMF content in strawberry juices.

Variables	n	COND	O ₂	BI	HMF
Storage		$p \leq 0.01^{\dagger}$	$p \leq 0.01^{\dagger}$	$p \leq 0.01^{\dagger}$	$p \leq 0.01^{\dagger}$
0 days	18	2733.4±20.7 ^b	7.69±0.08 ^a	1.51±0.01 ^b	4.43±0.04 ^b
7 days	18	3302.4±20.7 ^a	5.84±0.08 ^b	1.61±0.01 ^a	4.79±0.04 ^a
Average	36	3017.9±20.7	6.77±0.08	1.56±0.01	4.61±0.04
PEF		$p \leq 0.01^{\dagger}$	$p \leq 0.01^{\dagger}$	$p = 0.66^{\ddagger}$	$p = 0.33^{\ddagger}$
1.5 min	12	2725.2±25.3 ^c	7.05±0.10 ^a	1.57±0.01 ^a	4.63±0.04 ^a
3 min	12	3054.1±25.3 ^b	6.87±0.10 ^a	1.56±0.01 ^a	4.64±0.04 ^a
4.5 min	12	3274.6±25.3 ^a	6.38±0.10 ^a	1.56±0.01 ^a	4.55±0.04 ^a
Average	36	3018.0±25.3	6.77±0.10	1.56±0.01	4.61±0.04
HPU		$p \leq 0.01^{\dagger}$	$p \leq 0.01^{\dagger}$	$p \leq 0.01^{\dagger}$	$p = 0.29^{\ddagger}$
2.5 min	12	2754.2±25.3 ^c	6.96±0.10 ^a	1.57±0.01 ^a	4.63±0.04 ^a
5 min	12	2987.1±25.3 ^b	6.86±0.10 ^a	1.59±0.01 ^a	4.65±0.04 ^a
7.5 min	12	3312.6±25.3 ^a	6.47±0.10 ^b	1.52±0.01 ^b	4.55±0.04 ^a
Average	36	3018.0±25.3	6.76±0.10	1.56±0.01	4.61±0.04
PEF + HPU		$p \leq 0.01^{\dagger}$	$p = 0.23^{\ddagger}$	$p \leq 0.01^{\dagger}$	$p = 0.06^{\ddagger}$
1.5 min + 2.5 min	12	2174.8±13.9 ^c	7.14±0.2 ^a	1.64±0.02 ^a	4.77±0.07 ^a
1.5 min + 5 min	12	2838.3±13.9 ^b	7.27±0.2 ^a	1.59±0.02 ^a	4.64±0.07 ^a
1.5 min + 7.5 min	12	3162.5±13.9 ^a	6.73±0.2 ^a	1.47±0.02 ^b	4.49±0.07 ^a
Average	36	2725.2±43.9	7.05±0.21	1.57±0.02	4.63±0.07
PEF + HPU		$p \leq 0.01^{\dagger}$	$p = 0.42^{\ddagger}$	$p \leq 0.01^{\dagger}$	$p = 0.07^{\ddagger}$
3 min + 2.5 min	12	3156.5±35.5 ^a	7.02±0.14 ^a	1.60±0.01 ^a	4.68±0.08 ^a
3 min+5 min	12	2740.5±35.5 ^b	6.75±0.14 ^a	1.60±0.01 ^a	4.77±0.08 ^a
3 min+7.5 min	12	3265.2±35.5 ^a	6.85±0.14 ^a	1.47±0.01 ^b	4.47±0.08 ^a
Average	36	3054.1±35.5	6.87±0.14	1.56±0.01	4.64±0.08
PEF+HPU		$p \leq 0.01^{\dagger}$	$p \leq 0.01^{\dagger}$	$p \leq 0.01^{\dagger}$	$p = 0.17^{\ddagger}$
4.5 min+2.5 min	12	2931.3±43.9 ^b	6.74±0.15 ^a	1.48±0.02 ^b	4.44±0.09 ^a
4.5 min+5 min	12	3382.5±43.9 ^a	6.57±0.15 ^a	1.58±0.02 ^a	4.53±0.09 ^a
4.5 min+7.5 min	12	3510.0±43.9 ^a	5.85±0.15 ^b	1.61±0.02 ^a	4.70±0.09 ^a
Average	36	3274.6±43.9	6.39±0.15	1.56±0.02	4.56±0.09

The results are expressed as mean ± standard error. Values represented with different letters are statistically different at $p \leq 0.05$;

[†]significant factor in multifactor analysis.

[‡]not significant factor in multifactor analysis.

COND, conductivity (μS/cm); O₂-oxygen content (mg/L); BI, browning index; HMF, hydroxymethylfurfural (mg/L).

browning. The reduced amount of dissolved oxygen in the juices during storage could be result of oxidation, as shown by the results of storage-induced changes in color parameters (Enaru *et al.*, 2021).

The results also show that the processing time for the PEF and HPU treatments significantly influences the conductivity, with higher values achieved with longer processing times. An exception is the treatment with PEF (3 min) + HPU (2.5 min), where significantly higher conductivity values were obtained, as well as the treatment with the longest processing time (PEF 3 min + HPU 7.5 min). A longer treatment time for both technologies and their combination leads to a more effective electroporation of the cell membrane and, consequently, an easier efflux of cell material into the extracellular space, which may be associated with osmotic flow and a redistribution of moisture within the sample (Lebovka *et al.*, 2001). The results obtained are consistent with the findings of Zou & Jiang (2016), where conductivity values increased with the increasing duration of ultrasonic treatment of carrot juice.

Oxygen in juices can lead to negative changes in anthocyanin content due to oxidation and can also negatively affect color stability (Buvé *et al.*, 2018). Storage significantly affected oxygen reduction in the juice samples, which is consistent with previous reports (Solomon *et al.*, 1995). In most cases, longer PEF, HPU, and PEF+HPU treatments reduced oxygen content in the samples. PEF treatment allows easier removal of air from the tissue due to the electroporation and improved permeability of cell membranes (Trusinska *et al.*, 2023). Ultrasound can also be successfully used to degas juices and other fluids (Khan *et al.*, 2020). Dissolved gasses, such as oxygen, can act as nuclei and form bubbles, which float to the surface and can be removed from the juice, i.e., the treated liquid (Zenker *et al.*, 2003). The results obtained show that lower dissolved oxygen concentrations were observed at shorter PEF (1.5 and 3 min) and HPU (2.5 and 5 min) processing times. Interestingly, when the technologies were combined, the processing time had no significant effect on the dissolved oxygen content, except for the longest processing times where the minimum dissolved oxygen was observed at PEF 4.5 min + HPU 7.5 min. This confirms that the synergistic effect of PEF and HPU technologies is most effective in reducing dissolved oxygen at longer processing times, allowing greater stability of strawberry juices during storage.

Color plays an important role for consumers when selecting strawberry products, and the development of undesirable browning was determined by the browning index. The storage of strawberry juices significantly influenced the development of brown coloration; stored juices had higher BI values than non-stored juices. Brown coloration

can be caused by factors such as storage temperature, light, oxygen, pH, metals, and enzymes (Holzwarth *et al.*, 2011; Holzwarth *et al.*, 2012). As shown in Table 4, dissolved oxygen decreased during storage, making oxidation processes less likely. The most likely cause of the development of an undesirable brown color is enzymatic. Since samples were treated with non-thermal technologies, this excluded the use of high temperatures in the pre-treatment that are commonly used for enzymatic inactivation in juice production. Hence, it is likely that active enzymes caused these undesirable changes. This has already been confirmed in a study where thermal treatment (75°C/20 min) had the strongest impact on the inactivation of polyphenol oxidase compared to PEF and thermosonification in orange juice (Sulaiman *et al.*, 2016). In addition, ultrasonic treatment can cause an increase in polyphenol oxidase activity (Bi *et al.*, 2015). Considering the influence of different treatment times, BI was found to be more pronounced in juices treated with shorter treatment times. The only exception was an increase in BI during the longest treatment with a combination of PEF (4.5 min) + HPU (7.5 min). These results suggest that there is a limit beyond which prolonged treatment time with the combination of PEF+HPU technologies will cause the loss of red color, which could be related to the degradation of anthocyanins.

When examining the HMF content, the results show that only storage significantly influences the formation of HMF, with a higher concentration found in juices stored for 7 days compared to those stored for 0 days. These results align well with various studies that reported a proportional increase in HMF content during storage and when exposed to higher temperature (Singh & Sharma, 2017). The PEF and HPU technologies with different treatment times, had no significant effect on the HMF content of the treated juice samples. The findings are consistent with literature data that PEF technology had no effect on HMF formation in orange juice (Agcam *et al.*, 2016), in sour cherry juice (Akdemir Evrendilek *et al.*, 2021) and in apple juice (Akdemir Evrendilek *et al.*, 2016). Compared to those stored for 0 days. These results align well with various studies that reported a proportional increase in HMF content during storage and when exposed to higher temperatures (Singh & Sharma, 2017). The PEF and HPU technologies, with different treatment times, had no significant effect on the HMF content of the treated juice samples. These findings are consistent with literature data, which indicate that PEF technology had no effect on HMF formation in orange juice (Agcam *et al.*, 2016), sour cherry juice (Akdemir Evrendilek *et al.*, 2021), and apple juice (Akdemir Evrendilek *et al.*, 2016). This trend could be related to the high retention of ascorbic acid in juices, which, if not oxidized, cannot provide reactive carbonyl groups that can act as precursors for non-enzymatic browning reactions (Akdemir Evrendilek *et al.*, 2016).

The influence of pulsed electric field (PEF) in combination with high-power ultrasound (HPU) and on the anthocyanins and color stability of strawberry juices during storage

The average anthocyanin content of the treated strawberry juices (17.37 ± 0.08 mg/100 mL) is consistent with previous results (Bebek Markovinović *et al.*, 2022a; Bebek Markovinović *et al.*, 2022b) (Table 4). The storage of strawberry juice led to a significant increase (6.1%) in the anthocyanin content. The same trend was observed in an earlier study (Bebek Markovinović *et al.*, 2022c), in which strawberry juices treated with PEF during a 7-day storage period showed a 7.7% increase in anthocyanin

content. The positive effect of storage on anthocyanin content can be explained by its subsequent extraction, i.e., the release of intracellular compounds from damaged cells during storage.

When considering the influence of processing time during PEF treatment on the stability of anthocyanins, the greatest stability was observed at a medium PEF treatment time of 3 minutes. In contrast, anthocyanins were preserved to a lesser extent at both short and long PEF treatment times, indicating an inflection point in the data. Literature reports that the content of anthocyanins in strawberry juices depends significantly on the duration of the PEF treatment and the strength of the electric

Table 4. Influence of storage, PEF and HPU treatments on the anthocyanins and color parameters.

Variables	n	ANT	L*	a*	b*	C*	H*	ΔE^*_{ab}
Storage		$p \leq 0.01^\dagger$	$p \leq 0.01^\dagger$	$p \leq 0.01^\dagger$	$p \leq 0.01^\dagger$	$p \leq 0.01^\dagger$	$p \leq 0.01^\dagger$	$p \leq 0.01^\dagger$
0 days	18	17.72 ± 0.04^b	31.48 ± 0.01^a	18.64 ± 0.03^a	7.96 ± 0.01^a	20.27 ± 0.03^a	23.08 ± 0.04^a	2.85 ± 0.06^b
7 days	18	18.81 ± 0.04^a	30.87 ± 0.01^b	17.77 ± 0.03^b	7.16 ± 0.01^b	19.16 ± 0.03^b	21.95 ± 0.04^b	4.11 ± 0.06^a
Average	36	18.27 ± 0.04	31.18 ± 0.01	18.21 ± 0.03	7.56 ± 0.01	19.72 ± 0.03	22.52 ± 0.04	3.48 ± 0.06
PEF		$p \leq 0.01^\dagger$	$p \leq 0.01^\dagger$	$p \leq 0.01^\dagger$	$p \leq 0.01^\dagger$	$p \leq 0.01^\dagger$	$p \leq 0.01^\dagger$	$p \leq 0.01^\dagger$
1.5 min	12	18.49 ± 0.05^b	30.78 ± 0.01^c	17.76 ± 0.03^c	7.21 ± 0.01^c	19.17 ± 0.03^c	22.09 ± 0.05^c	4.13 ± 0.07^a
3 min	12	18.93 ± 0.05^a	31.24 ± 0.01^b	18.29 ± 0.03^b	7.66 ± 0.01^b	19.83 ± 0.03^b	22.66 ± 0.05^a	3.34 ± 0.07^b
4.5 min	12	17.37 ± 0.05^c	31.50 ± 0.01^a	18.57 ± 0.03^a	7.82 ± 0.01^a	20.16 ± 0.03^a	22.80 ± 0.05^b	2.96 ± 0.07^c
Average	36	18.26 ± 0.05	31.17 ± 0.01	18.21 ± 0.03	7.56 ± 0.01	19.72 ± 0.03	22.52 ± 0.05	3.48 ± 0.07
HPU		$p \leq 0.01^\dagger$	$p \leq 0.01^\dagger$	$p \leq 0.01^\dagger$	$p \leq 0.01^\dagger$	$p \leq 0.01^\dagger$	$p = 0.02^\dagger$	$p \leq 0.01^\dagger$
2.5 min	12	18.12 ± 0.05^b	31.17 ± 0.01^b	18.39 ± 0.03^a	7.62 ± 0.01^a	19.91 ± 0.03^a	22.48 ± 0.05^b	3.30 ± 0.07^b
5 min	12	19.03 ± 0.05^a	31.10 ± 0.01^c	18.02 ± 0.03^c	7.46 ± 0.01^b	19.50 ± 0.03^c	22.43 ± 0.05^b	3.71 ± 0.07^a
7.5 min	12	17.64 ± 0.05^c	31.25 ± 0.01^a	18.21 ± 0.03^b	7.61 ± 0.01^a	19.74 ± 0.03^b	22.63 ± 0.05^a	3.43 ± 0.07^b
Average	36	18.26 ± 0.05	31.17 ± 0.01	18.21 ± 0.03	7.56 ± 0.01	19.72 ± 0.03	22.51 ± 0.05	3.48 ± 0.07
PEF+HPU		$p \leq 0.01^\dagger$	$p \leq 0.01^\dagger$	$p \leq 0.01^\dagger$	$p \leq 0.01^\dagger$	$p \leq 0.01^\dagger$	$p \leq 0.01^\dagger$	$p \leq 0.01^\dagger$
1.5 min+2.5 min	12	19.01 ± 0.06^a	30.91 ± 0.02^a	18.34 ± 0.04^a	7.47 ± 0.02^a	19.80 ± 0.04^a	22.14 ± 0.04^a	3.50 ± 0.14^b
1.5 min+5 min	12	18.51 ± 0.06^b	30.64 ± 0.02^c	17.36 ± 0.04^c	6.98 ± 0.02^c	18.71 ± 0.04^c	21.89 ± 0.04^b	4.61 ± 0.14^a
1.5 min+7.5 min	12	17.96 ± 0.06^c	30.80 ± 0.02^b	17.58 ± 0.04^b	7.18 ± 0.02^b	18.99 ± 0.04^b	22.22 ± 0.04^a	4.28 ± 0.14^a
Average	36	18.49 ± 0.06	30.78 ± 0.02	17.76 ± 0.04	7.21 ± 0.02	19.17 ± 0.04	22.08 ± 0.04	4.13 ± 0.14
PEF+HPU		$p = 0.05^\dagger$	$p \leq 0.01^\dagger$	$p \leq 0.01^\dagger$	$p \leq 0.01^\dagger$	$p \leq 0.01^\dagger$	$p \leq 0.01^\dagger$	$p \leq 0.01^\dagger$
3 min+2.5 min	12	18.71 ± 0.10^b	31.01 ± 0.04^b	17.92 ± 0.03^a	7.38 ± 0.02^b	19.38 ± 0.04^b	22.34 ± 0.04^b	3.84 ± 0.10^a
3 min+5 min	12	19.17 ± 0.10^a	31.01 ± 0.04^b	17.81 ± 0.03^b	7.38 ± 0.02^b	19.28 ± 0.04^b	22.45 ± 0.04^b	3.93 ± 0.10^a
3 min+7.5 min	12	$18.90 \pm 0.10^{a,b}$	31.71 ± 0.04^a	19.14 ± 0.03^c	8.22 ± 0.02^a	20.83 ± 0.04^a	23.18 ± 0.04^a	2.26 ± 0.10^b
Average	36	18.93 ± 0.10	31.24 ± 0.04	18.29 ± 0.03	7.66 ± 0.02	19.83 ± 0.04	22.66 ± 0.08	3.34 ± 0.10
PEF+HPU		$p \leq 0.01^\dagger$	$p \leq 0.01^\dagger$	$p \leq 0.01^\dagger$	$p \leq 0.01^\dagger$	$p \leq 0.01^\dagger$	$p \leq 0.01^\dagger$	$p \leq 0.01^\dagger$
4.5 min+2.5 min	12	16.64 ± 0.08^b	31.60 ± 0.02^a	18.91 ± 0.09^a	8.02 ± 0.02^a	20.55 ± 0.08^b	22.96 ± 0.13^a	2.55 ± 0.13^b
4.5 min+5 min	12	19.42 ± 0.08^a	31.65 ± 0.02^a	18.88 ± 0.09^a	8.02 ± 0.02^a	20.52 ± 0.08^b	22.96 ± 0.13^a	2.59 ± 0.13^b
4.5 min+7.5 min	12	16.05 ± 0.08^c	31.25 ± 0.02^b	17.93 ± 0.09^b	7.42 ± 0.02^b	19.40 ± 0.08^a	22.49 ± 0.13^b	3.74 ± 0.13^a
Average	36	17.37 ± 0.08	31.50 ± 0.02	18.57 ± 0.09	7.82 ± 0.02	20.16 ± 0.08	22.80 ± 0.13	2.96 ± 0.13

The results are expressed as mean $\pm$ standard error. Values represented with different letters are statistically different at $p \leq 0.05$; † significant factor in multifactor analysis;

* not significant factor in multifactor analysis.

ANT, monomeric anthocyanins (mg/100 g); L*, lightness; a*, b*, CIELab coordinates; C*, chroma; H*, hue; ΔE^*_{ab} , color change.

field, with shorter treatments and higher electric field strengths leading to greater preservation of anthocyanins (Odriozola-Serrano *et al.*, 2008).

In contrast, in the case of HPU, a treatment duration of 5 min resulted in the highest anthocyanin stability, while a treatment duration of 7.5 minutes resulted in the lowest anthocyanin stability. This is consistent with previous results, where a significant effect of treatment duration was found on the reduction of anthocyanin content in HPU-treated strawberry juices (Tiwari *et al.*, 2008). The increase in anthocyanin content with shorter treatment times could be explained by the improved extraction of pigments from the suspended pulp.

The combined PEF and HPU treatments at different durations significantly influenced the stability of the anthocyanins. With shorter exposures to PEF (1.5 min) and HPU (2.5 min), the stability of the anthocyanins was better. However, as the processing time in PEF technology increased (3 min and 4.5 min), longer processing times in HPU technology (5 min and 7.5 min) were required for higher stability of the anthocyanins. This confirms the synergistic influence of the combination of PEF and HPU on the stability of the anthocyanins. Additionally, the combination of 4.5 min PEF + 5 min HPU showed the best effect on the stability of the anthocyanins.

Storage at 4°C for 7 days significantly reduced the lightness (L^*), suggesting that storage contributed to the darkening of the juices. Considering that the BI increased significantly and the HMF content decreased during storage (Table 4), the changes in L^* values can most likely be attributed to the previously mentioned enzymatic activity (Bi *et al.*, 2015; Sulaiman *et al.*, 2016). Furthermore, these results are consistent with the findings of Tiwari *et al.* (2009), who also observed decreased L^* values in orange juice after 7 days of storage at 10°C.

By extending the PEF treatment duration, the L^* value increases significantly. In contrast, a different trend was observed when extending the duration of the single HPU treatment. Extending the HPU treatment up to 5 min resulted in a significant decrease in L^* , while extending the treatment up to 7.5 min caused an increase in L^* . Similar findings were previously reported, where L^* increased with ultrasound treatment of carrot juice up to a 20 min treatment duration, after which L^* stagnated (Zou & Jiang, 2016). In the combination of PEF (1.5 min) and HPU treatments, an increase in HPU treatment duration resulted in a significant decrease in L^* up to 5 min. In contrast, further treatment led to a significant increase, but not beyond the initial value. Additionally, a different trend was observed with PEF (3 min) and HPU treatment. No change in L^* was observed when the HPU treatment time was extended up to 5 min, after which L^*

increased significantly. The increase in L^* could be caused by the degradation of unstable particles during ultrasonic treatment, which affects the changes in color parameters (Tiwari *et al.*, 2009). In contrast to these results, the same trend was observed with the combination of PEF (4.5 min) and HPU treatment up to 5 min. After that, the opposite trend was observed, with the L^* value decreasing significantly as the duration increased to 7.5 min. This could be related to the sample being exposed to prolonged treatment, resulting in degradation and a reduction in anthocyanin content, with the anthocyanin value being significantly reduced after the PEF (4.5 min) + HPU (7.5 min) treatment.

The a^* and b^* values follow almost the same trend with respect to storage and the technologies used. Storage led to a significant decrease in both a^* and b^* values. These results are consistent with the color parameters of canned strawberries with added black carrot concentrate during the same storage period (Kammerer *et al.*, 2006). With increasing PEF treatment duration, there was a significant increase in both a^* and b^* values. In contrast, the individual HPU treatment showed a significant decrease in a^* and b^* values when the treatment duration was increased from 2.5 to 5 min. However, a further extension of the treatment to 7.5 min increased both a^* and b^* values. The combination of PEF (1.5 and 3 min) and HPU treatment showed the same trend as HPU treatment alone, with an increase in duration (the exception being PEF at 3 min) and HPU, with an increase in treatment time from 2.5 to 5 min. In the PEF (4.5 min) and HPU treatment, there was a significant decrease in the a^* value with increasing HPU treatment duration. In contrast, the b^* value remained unchanged with an increase in duration from 2.5 to 5 min and decreased significantly with further treatment (7.5 min). Color changes during sonication could be attributed to the influence of cavitation, which causes physical, chemical, and biological reactions that lead to the cleavage of certain particles, such as enzymes (Sala *et al.*, 1995).

Values of C^* (chroma) and H^* (hue) follow almost the same trend with respect to storage conditions and the application of different PEF and HPU treatment times, both individually and in combination. Storing the juices at 4°C for 7 days had a significant effect on the reduction of both C^* and H^* values. These results are consistent with previous reports (Kammerer *et al.*, 2006), where a decrease in these values was observed in canned strawberries after 4 weeks of storage. Increasing the PEF treatment duration significantly affected the increase in both values (C^* and H^*). In contrast, increasing the HPU treatment time from 2.5 to 5 min decreased both values, while further treatment caused a significant increase in these values. The combination of PEF (1.5 min) and HPU treatment showed the same trend in C^* and H^* values.

as HPU treatment alone. PEF (3 min) and HPU did not affect C^* and H^* levels when the HPU treatment time was increased up to 5 min, after which there was an increase in these two values. In contrast, PEF (4.5 min) and HPU treatment above 5 min showed a decrease in these values. These results are partially consistent with previous reports on colorimetric parameters in red and yellow watermelon juice sonicated for 4, 8, 12, and 16 min (Yıkımsı, 2020). The trends are similar, and the differences can be attributed to the varying treatment times and the intensity of the device.

ΔE^*_{ab} , i.e. the overall color difference between the untreated and treated samples, was evaluated to determine whether the PEF and HPU treatments produce a color change that the consumer can perceive. The mean values for ΔE^*_{ab} ranged from 2.96 ± 0.13 to 4.13 ± 0.14 . These were not large or very significant differences, hence the treated juices may be sensory acceptable in terms of color. However, when $\Delta E^*_{ab} = 1.5\text{--}3.0$, the color change is noticeable, and when $\Delta E^*_{ab} = 3.0\text{--}6.0$, the difference is considerable (Chen, 2008). Storage significantly affected the ΔE^*_{ab} value, which was significantly higher in stored samples. An increase in ΔE^* value after 4 weeks of storage of canned strawberries was also observed (Kammerer *et al.*, 2006). The PEF treatment reduced the ΔE^*_{ab} , while the application of the HPU treatment showed the opposite trend. Extending HPU treatment from 2.5 to 5 min led to an increase in ΔE^*_{ab} , while further treatment led to a decrease. The same trend was observed with the combination of PEF (1.5 min) and HPU treatment. PEF (3 min) and HPU did not affect ΔE^*_{ab} values when the HPU treatment time was increased to 5 min, after which there was an increase in ΔE^*_{ab} value. In contrast, when using PEF (4.5 min) and HPU treatment for 5 min of the other treatment technology, a decrease in ΔE^*_{ab} value was observed. Overall, the values of ΔE^*_{ab} were below 6, which indicates that the differences in color were acceptable, i.e., appreciable (Chen, 2008).

Optimization pulsed electric field (PEF) and high-power ultrasound (HPU) processing parameters

In order to obtain products with the best nutritional and/or biological value, as well as the best physicochemical properties, it is important to optimize the process parameters of the technologies. Table 5 shows the optimal parameters for the PEF and HPU treatments that led to maximum anthocyanin yield and minimum changes in color, conductivity, BI, and HMF content. As mentioned above, longer storage favors the content of anthocyanins, with a yield of 20.29 mg/100 g of these polyphenols on the 7th day of storage, achieved with PEF and HPU treatment durations of 2.19 and 7.48 minutes, respectively. On the other hand, if the combined technology aims to minimize

conductivity and browning, the samples should not be stored at all (i.e., storage should be 0 days), and the duration of the PEF treatment should be relatively short, no longer than 1.5 minutes. The optimal values for the lowest conductivity (2156.7 $\mu\text{S}/\text{cm}$) and browning index (1.13) should be achieved with 2.5 and 7.5 minutes of HPU treatment, respectively. The final color change was achieved with 3.14 minutes of PEF and 7.5 minutes of HPU.

Based on the parameters studied, and if PEF was used as the initial treatment followed by HPU in this hurdle concept, it was more practical to shorten the PEF treatment and prolong the HPU treatment to achieve optimal nutritional value and the least adverse effects on the samples. There is no data in the literature regarding the optimization of the parameters for the combination of PEF and HPU technologies for anthocyanin preservation. In a previous study, the authors compared the yield of anthocyanins from blueberry by-products using PEF treatment (5–30 kV/cm, 1000–3000 Hz) and ultrasonic treatment (125 W, 60 min, 40°C). It was shown that PEF treatment is significantly more effective and requires a shorter treatment time and lower temperatures than sonication (Zhou *et al.*, 2015). These results are consistent with our findings, where the optimization of the parameters of the combined PEF and HPU technologies showed that a shorter PEF treatment than HPU was required to achieve the best anthocyanin yield and the least changes in color and HMF content.

Conclusions

This work aimed to investigate the influence of pulsed electric field (PEF) and high-power ultrasound (HPU) treatment at different operating times on anthocyanin content, color parameters, conductivity, dissolved oxygen, HMF content, and browning index in strawberry juices stored at 4°C for 7 days using a chemometric approach. First, the untreated (controls) samples were

Table 5. Optimal processing parameters for the combination of PEF and HPU technologies to achieve maximum monomeric anthocyanin content and minimum changes in color, conductivity, browning index, and HMF content.

Parameter	ANT	COND	BI	HMF	ΔE^*_{ab}
Storage (days)	7.00	0.00	0.00	7.00	0.00
PEF treatment (min)	2.19	1.50	1.50	3.77	3.14
HPU treatment (min)	7.48	2.50	7.50	2.50	7.50
Optimum	20.29	2156.7	1.13	4.17	1.53

ANT, monomeric anthocyanins (mg/100 g); COND, conductivity ($\mu\text{S}/\text{cm}$); BI, browning index; HMF, hydroxymethylfurfural (mg/L); ΔE^*_{ab} , color change. PEF, pulsed electric field (30 kV/cm, 100 Hz); HPU, high-power ultrasound (amplitude 25%, pulse 50%).

compared with the treated samples (PEF + HPU), and it was found that they differed in terms of conductivity and color parameters. Conductivity was higher in the treated samples than in the control samples, as was the overall color change ΔE^*_{ab} . A storage time of 7 days at 4°C had a significant effect on the increase in anthocyanin concentration, conductivity, browning index, HMF content, and total color change ΔE^*_{ab} , while the opposite was true for the dissolved oxygen and CIEL*a*b* parameters. The highest anthocyanin stability was observed in the samples treated with PEF (3 min) and HPU technology (2.5, 5, and 7.5 min). An increase in treatment time led to increased conductivity without any effect on the formation of hydroxymethylfurfural. The order and length of exposure mattered in the sense that shorter PEF treatments followed by HPU led to decreased browning, while the inverse treatment (i.e., the most intensive PEF treatment followed by HPU) increased it. PEF and HPU treatments showed good effects in reducing dissolved oxygen in the juices. Moreover, the color changes of the treated samples ranged from noticeable to considerable, which was still within the acceptable limits. All these results indicate that the PEF and HPU technologies are suitable for the production of strawberry-based functional juices as they help to preserve their biological and nutritional value and properties.

The optimal parameters for combining PEF and HPU treatments to obtain strawberry juice with the highest yield of anthocyanins were a short 2.19 min for PEF treatment followed by a 7.48 min HPU treatment during a 7-day storage period. In general, shorter PEF treatment times and longer HPU treatment times favored better anthocyanins yield and minimal color changes, browning index, and hydroxymethylfurfural formation. In summary, the combination of PEF and HPU technologies has the potential to preserve the nutritional and biological value of strawberry juices, prevent the formation of hydroxymethylfurfural, and preserve the physicochemical properties of the native product.

Author Contributions

Conceptualization, D.B.K., P.P., A.M.K.; methodology, D.B.K., V.S., T.J., and B.P.; software, P.P.; validation, A.B.M., S.M., B.P., and Z.H.; formal analysis, A.B.M., V.S., T.J., B.P., and S.M.; investigation, A.B.M., V.S., T.J., B.P., and S.M.; resources, D.B.K. and Z.H.; data curation, P.P.; writing—original draft preparation, A.B.M., D.B.K., and P.P.; writing—review and editing, V.S., T.J., B.P., S.M., Z.H., and A.M.K.; visualization, A.B.M., P.P., S.M., B.P. and T.J.; supervision, D.B.K.; project administration, D.B.K. and Z.H.; funding acquisition, D.B.K. and A.M.K. All authors have read and agreed to the published version of the manuscript.

Conflict of Interest

The authors declare no conflict of interest.

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References

- Agcam, E., Akyildiz, A., & Akdemir Evrendilek, G. (2016). A comparative assessment of long-term storage stability and quality attributes of orange juice in response to pulsed electric fields and heat treatments. *Food and Bioproducts Processing*, 99, 90–98. <https://doi.org/10.1016/j.fbp.2016.04.006>
- Akdemir Evrendilek, G., Agcam, E., & Akyildiz, A. (2021). Effects of pulsed electric fields on sour cherry juice properties and formations of furfural and hydroxymethylfurfural. *International Journal of Food Engineering*, 17(3), 217–226. <https://doi.org/10.1515/ijfe-2020-0189>
- Akdemir Evrendilek, G., Celik, P., Agcam, E., & Akyildiz, A. (2016). Assessing impacts of pulsed electric fields on quality attributes and furfural and hydroxymethylfurfural formations in apple juice. *Journal of Food Process Engineering*, 40(5), e12524. <https://doi.org/10.1111/jfpe.12524>
- Official methods of analysis: Changes in official methods of analysis made at the annual meeting, (1990).
- Barba, E., Putnik, P., & Kovačević, D. B. (2020). *Agri-food industry strategies for healthy diets and sustainability : New challenges in nutrition and public health*. Elsevier, Academic Press, eBook ISBN: 9780128172278.
- Bebek Markovinović, A., Brdar, D., Putnik, P., Bosiljkov, T., Durgo, K., Hudek Turković, A., et al. (2024a). Strawberry tree fruits (*Arbutus unedo* L.): Bioactive composition, cellular antioxidant activity, and 3D printing of functional foods. *Food Chemistry*, 433, e137287. <https://doi.org/10.1016/j.foodchem.2023.137287>
- Bebek Markovinović, A., Putnik, P., Bičanić, P., Brdar, D., Duralija, B., Pavlić, B., et al. (2022a). A chemometric investigation on the functional potential in high power ultrasound (HPU) processed strawberry juice made from fruits harvested at two stages of ripeness. *Molecules*, 28(1), e138. <https://doi.org/10.3390/molecules28010138>

- Bebek Markovinović, A., Putnik, P., Bosiljkov, T., Kostelac, D., Frece, J., Markov, K., et al. (2023). 3D Printing of Functional Strawberry Snacks: Food Design, Texture, Antioxidant Bioactive Compounds, and Microbial Stability. *Antioxidants*, 12(2), e436. <https://doi.org/10.3390/antiox12020436>
- Bebek Markovinović, A., Putnik, P., Duralija, B., Krivohlavek, A., Ivešić, M., Mandić Andačić, I., et al. (2022b). Chemometric Valorization of Strawberry (*Fragaria x ananassa* Duch.) cv. 'Albion' for the Production of Functional Juice: The Impact of Physicochemical, Toxicological, Sensory, and Bioactive Value. *Foods*, 11(5), e640. <https://doi.org/10.3390/foods11050640>
- Bebek Markovinović, A., Putnik, P., Stulić, V., Batur, L., Duralija, B., Pavlič, B., et al. (2022c). The application and optimization of HIPEF technology in the processing of juice from strawberries harvested at two stages of ripeness. *Foods*, 11(14), e1997. <https://doi.org/10.3390/foods11141997>
- Bebek Markovinović, A., Stulić, V., Putnik, P., Bekavac, N., Pavlič, B., Milošević, S., et al. (2024b). High-Power Ultrasound (HPU) and Pulsed Electric Field (PEF) in the Hurdle Concept for the Preservation of Antioxidant Bioactive Compounds in Strawberry Juice—A Chemometric Evaluation—Part II. *Foods*, 13(4), e537. <https://doi.org/10.3390/foods13040537>
- Bi, X., Hemar, Y., Balaban, M. O., & Liao, X. (2015). The effect of ultrasound on particle size, color, viscosity and polyphenol oxidase activity of diluted avocado puree. *Ultrasonics Sonochemistry*, 27, 567–575. <https://doi.org/10.1016/j.ultsonch.2015.04.011>
- Bursać Kovačević, D., Putnik, P., Dragović-Uzelac, V., Pedisić, S., Režek Jambrak, A., & Herceg, Z. (2016). Effects of cold atmospheric gas phase plasma on anthocyanins and color in pomegranate juice. *Food Chemistry*, 190, 317–323. <https://doi.org/10.1016/j.foodchem.2015.05.099>
- Buvé, C., Kebede, B. T., De Batselier, C., Carrillo, C., Pham, H. T. T., Hendrickx, M., et al. (2018). Kinetics of colour changes in pasteurised strawberry juice during storage. *Journal of Food Engineering*, 216, 42–51. <https://doi.org/10.1016/j.jfoodeng.2017.08.002>
- Chen, J.-y., Du, J., Li, M.-l., & Li, C.-m. (2020). Degradation kinetics and pathways of red raspberry anthocyanins in model and juice systems and their correlation with color and antioxidant changes during storage. *LWT*, 128, e109448. <https://doi.org/10.1016/j.lwt.2020.109448>
- Chen, X. D. (2008). Food drying fundamentals. In X. D. Chen & A. S. Mujumdar (Eds.), *Drying Technologies in Food Processing* (pp. 1–55). Blackwell Publishing.
- Cohen, E., Birk, Y., Mannheim, C. H., & Saguy, I. S. (1998). A rapid method to monitor quality of apple juice during thermal processing. *LWT - Food Science and Technology*, 31(7–8), 612–616. <https://doi.org/10.1006/fstl.1998.0385>
- Cosme, F., Pinto, T., Aires, A., Morais, M. C., Bacelar, E., Anjos, R., ... Gonçalves, B. (2022). Red fruits composition and their health benefits—A review. *Foods*, 11(5), e644. <https://doi.org/10.3390/foods11050644>
- Crecente-Campo, J., Nunes-Damaceno, M., Romero-Rodríguez, M. A., & Vázquez-Odériz, M. L. (2012). Color, anthocyanin pigment, ascorbic acid and total phenolic compound determination in organic versus conventional strawberries (*Fragaria x ananassa* Duch, cv Selva). *Journal of Food Composition and Analysis*, 28(1), 23–30. <https://doi.org/10.1016/j.jfca.2012.07.004>
- Cserhalmi, Z., Sass-Kiss, Á., Tóth-Markus, M., & Lechner, N. (2006). Study of pulsed electric field treated citrus juices. *Innovative Food Science & Emerging Technologies*, 7(1–2), 49–54. <https://doi.org/10.1016/j.ifset.2005.07.001>
- Dixit, Y., O'Sullivan, C., Cullen, P. J., & Tiwari, B. K. (2018). Hurdle technologies for fruit juices. In *Fruit Juices* (pp. 539–554). <https://doi.org/10.1016/b978-0-12-802230-6.00027-8>
- Djekić, I., Velebit, B., Pavlič, B., Putnik, P., Šojić Merkulov, D., Bebek Markovinović, A., & Bursać Kovačević, D. (2023). Food Quality 4.0: Sustainable Food Manufacturing for the Twenty-First Century. *Food Engineering Reviews*, 15(4), 577–608. <https://doi.org/10.1007/s12393-023-09354-2>
- Enaru, B., Dreţcanu, G., Pop, T. D., Stănilă, A., & Diaconeasa, Z. (2021). Anthocyanins: Factors affecting their stability and degradation. *Antioxidants*, 10(12), e1967. <https://doi.org/10.3390/antiox10121967>
- Galanakis, C. M. (2021). Functionality of food components and emerging technologies. *Foods*, 10(1), e128. <https://doi.org/10.3390/foods10010128>
- Gharibzadeh, S. M. T., Hernández-Ortega, C., Welti-Chanes, J., Putnik, P., Barba, F. J., Mallikarjunan, K., ... Roohinejad, S. (2019). High pressure processing of food-grade emulsion systems: Antimicrobial activity, and effect on the physicochemical properties. *Food Hydrocolloids*, 87, 307–320. <https://doi.org/10.1016/j.foodhyd.2018.08.012>
- Gladikostić, N., Ikonić, B., Teslić, N., Zeković, Z., Božović, D., Putnik, P., et al. (2023). Essential Oils from Apiaceae, Asteraceae, Cupressaceae and Lamiaceae Families Grown in Serbia: Comparative Chemical Profiling with In Vitro Antioxidant Activity. *Plants*, 12(4), e745. <https://doi.org/10.3390/plants12040745>
- Graybill, P. M., & Davalos, R. V. (2020). Cytoskeletal Disruption after Electroporation and Its Significance to Pulsed Electric Field Therapies. *Cancers*, 12(5), e1132. <https://doi.org/10.3390/cancers12051132>
- Guerrero-Beltrán, J. Á., & Welti-Chanes, J. (2016). Pulsed Electric Fields. In B. Caballero, P. M. Finglas, & F. Toldrá (Eds.), *Encyclopedia of Food and Health* (pp. 561–565). Elsevier. <https://doi.org/10.1016/b978-0-12-384947-2.00579-1>
- Holzwarth, M., Korhummel, S., Carle, R., & Kammerer, D. R. (2011). Impact of enzymatic mash maceration and storage on anthocyanin and color retention of pasteurized strawberry purées. *European Food Research and Technology*, 234(2), 207–222. <https://doi.org/10.1007/s00217-011-1601-y>
- Holzwarth, M., Korhummel, S., Kammerer, D. R., & Carle, R. (2012). Thermal inactivation of strawberry polyphenoloxidase and its impact on anthocyanin and color retention in strawberry (*Fragaria x ananassa* Duch.) purées. *European Food Research and Technology*, 235(6), 1171–1180. <https://doi.org/10.1007/s00217-012-1852-2>
- Kammerer, D. R., Schillmöller, S., Maier, O., Schieber, A., & Carle, R. (2006). Colour stability of canned strawberries using black

- carrot and elderberry juice concentrates as natural colourants. *European Food Research and Technology*, 224(6), 667–679. <https://doi.org/10.1007/s00217-006-0356-3>
- Khan, S. A., Dar, A. H., Bhat, S. A., Fayaz, J., Makroo, H. A., & Dwivedi, M. (2020). High intensity ultrasound processing in liquid foods. *Food Reviews International*, 38(6), 1123–1148. <https://doi.org/10.1080/87559129.2020.1768404>
- Lebovka, N. I., Bazhal, M. I., & Vorobiev, E. (2001). Pulsed electric field breakage of cellular tissues: visualisation of percolative properties. *Innovative Food Science & Emerging Technologies*, 2(2), 113–125. [https://doi.org/10.1016/s1466-8564\(01\)00024-8](https://doi.org/10.1016/s1466-8564(01)00024-8)
- Lebovka, N. I., Bazhal, M. I., & Vorobiev, E. (2002). Estimation of characteristic damage time of food materials in pulsed-electric fields. *Journal of Food Engineering*, 54(4), 337–346. [https://doi.org/10.1016/s0260-8774\(01\)00220-5](https://doi.org/10.1016/s0260-8774(01)00220-5)
- Martins, I. B. A., Oliveira, D., Rosenthal, A., Ares, G., & Deliza, R. (2019). Brazilian consumer's perception of food processing technologies: A case study with fruit juice. *Food Research International*, 125, e108555. <https://doi.org/10.1016/j.foodres.2019.108555>
- Maza, M. A., Pereira, C., Martínez, J. M., Camargo, A., Álvarez, I., & Raso, J. (2020). PEF treatments of high specific energy permit the reduction of maceration time during vinification of Caladoc and Grenache grapes. *Innovative Food Science & Emerging Technologies*, 63, e102375. <https://doi.org/10.1016/j.ifset.2020.102375>
- Odriozola-Serrano, I., Soliva-Fortuny, R., Gimeno-Añó, V., & Martín-Belloso, O. (2008). Kinetic study of anthocyanins, vitamin C, and antioxidant capacity in strawberry juices treated by high-intensity pulsed electric fields. *Journal of Agricultural and Food Chemistry*, 56(18), 8387–8393. <https://doi.org/10.1021/jf801537f>
- Putnik, P., Pavlič, B., Šojić, B., Zavadlav, S., Žuntar, I., Kao, L., ... Kovačević, D. B. (2020). Innovative hurdle technologies for the preservation of functional fruit juices. *Foods*, 9(6), e699. <https://doi.org/10.3390/foods9060699>
- Sala, F. J., Burgos, J., Condón, S., Lopez, P., & Raso, J. (1995). Effect of heat and ultrasound on microorganisms and enzymes. In *New Methods of Food Preservation* (pp. 176–204). https://doi.org/10.1007/978-1-4615-2105-1_9
- Singh, S. K., & Sharma, M. (2017). Review on biochemical changes associated with storage of fruit juice. *International Journal of Current Microbiology and Applied Sciences*, 6(8), 236–245. <https://doi.org/10.20546/ijcmas.2017.608.032>
- Solomon, O., Svanberg, U., & Sahlström, A. (1995). Effect of oxygen and fluorescent light on the quality of orange juice during storage at 8°C. *Food Chemistry*, 53(4), 363–368. [https://doi.org/10.1016/0308-8146\(95\)99828-n](https://doi.org/10.1016/0308-8146(95)99828-n)
- Sulaiman, A., Farid, M., & Silva, F. V. M. (2016). Quality stability and sensory attributes of apple juice processed by thermosonication, pulsed electric field and thermal processing. *Food Science and Technology International*, 23(3), 265–276. <https://doi.org/10.1177/1082013216685484>
- Tiwari, B. K., O'Donnell, C. P., Muthukumarappan, K., & Cullen, P. J. (2009). Effect of sonication on orange juice quality parameters during storage. *International Journal of Food Science & Technology*, 44(3), 586–595. <https://doi.org/10.1111/j.1365-2621.2008.01858.x>
- Tiwari, B. K., O'Donnell, C. P., Patras, A., Brunton, N., & Cullen, P. J. (2008). Stability of anthocyanins and ascorbic acid in sonicated strawberry juice during storage. *European Food Research and Technology*, 228(5), 717–724. <https://doi.org/10.1007/s00217-008-0982-z>
- Trusinska, M., Drudi, E., Rybak, K., Tylewicz, U., & Nowacka, M. (2023). Effect of the Pulsed Electric Field Treatment on Physical, Chemical and Structural Changes of Vacuum Impregnated Apple Tissue in Aloe Vera Juices. *Foods*, 12(21), e3957. <https://doi.org/10.3390/foods12213957>
- Warner, R., Wu, B.-S., MacPherson, S., & Lefsrud, M. (2021). A Review of strawberry photobiology and fruit flavonoids in controlled environments. *Frontiers in Plant Science*, 12, e611893. <https://doi.org/10.3389/fpls.2021.611893>
- Yıkmaş, S. (2020). Sensory, physicochemical, microbiological and bioactive properties of red watermelon juice and yellow watermelon juice after ultrasound treatment. *Journal of Food Measurement and Characterization*, 14(3), 1417–1426. <https://doi.org/10.1007/s11694-020-00391-7>
- Yildiz, S., Pokhrel, P. R., Unluturk, S., & Barbosa-Cánovas, G. V. (2021). Shelf life extension of strawberry juice by equivalent ultrasound, high pressure, and pulsed electric fields processes. *Food Research International*, 140, e110040. <https://doi.org/10.1016/j.foodres.2020.110040>
- Zenker, M., Heinz, V., & Knorr, D. (2003). Application of ultrasound-assisted thermal processing for preservation and quality retention of liquid foods. *Journal of Food Protection*, 66(9), 1642–1649. <https://doi.org/10.4315/0362-028x-66.9.1642>
- Zhang, M.-Q., Zhang, J., Zhang, Y.-T., Sun, J.-Y., Prieto, M. A., Simal-Gandara, J., ... Liu, C. (2023). The link between the phenolic composition and the antioxidant activity in different small berries: A metabolomic approach. *Lwt*, 182, e114853. <https://doi.org/10.1016/j.lwt.2023.114853>
- Zhou, Y., Zhao, X., & Huang, H. (2015). Effects of pulsed electric fields on anthocyanin extraction yield of blueberry processing by-products. *Journal of Food Processing and Preservation*, 39(6), 1898–1904. <https://doi.org/10.1111/jfpp.12427>
- Zou, Y., & Jiang, A. (2016). Effect of ultrasound treatment on quality and microbial load of carrot juice. *Food Science and Technology*, 36(1), 111–115. <https://doi.org/10.1590/1678-457x.0061>