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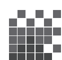

ORIGINAL SCIENTIFIC PAPER

Impact of heat-assisted HVED plasma treatment on quality of apple juice

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Abstract

High voltage electrical discharge (HVED) plasma processing receives more and more attention due to its potential to assure microbial safety and retain quality of treated products. The influence of combined thermal and high voltage electrical discharge plasma treatment on apple juice quality was investigated. Apple juice samples were treated under defined plasma treatment parameters of time (3, 6 and 9 min), frequency (60, 90 and 120 Hz) and temperature (30, 40 and 50 °C) in hybrid plasma reactor. Prior to treatment, juice samples were inoculated with Saccharomyces cerevisiae ATCC 204508 to investigate inactivation possibilities of plasma treatment on yeasts as common juice microorganisms. Quality parameters (pH, electrical conductivity, phenolic content and antioxidant activity) of treated and untreated apple juice were investigated and compared. Results have shown effectiveness of HVED plasma treatment in yeast inactivation up to 6.6 log_{10} in 9 min of treatment at 120 Hz and temperature of 40 °C. In treated samples there were no significant changes in phenolic content.

Keywords: *HVED plasma, apple juice, Saccharomyces cerevisiae, antioxidant activity*

Introduction

Traditionally, fruit juices are thermally pasteurized (Yi et al., 2017) due to effectiveness of pasteurization in microorganisms and enzymes inactivation which are responsible for food deterioration (Awuah, et al., 2007). However, thermal processing may affect colour, odour, flavour, texture, and health effect of juices (De Paepe et al., 2014) which opposes to consumers demands of natural and fresh fruit juices appearance and flavour (Yi et al., 2017). Fruit juices are highly susceptible to spoilage due to moulds, aciduric bacteria and yeasts presence of which predominating spoilers are yeasts because of their high acid tolerance and ability to grow anaerobically (Massini, et al., 2018. Scientists, in a field of food engineering are working on non-thermal food processes which will possibly replace traditional thermal treatments to minimize juice spoilage, but also the loss of quality attributes (Pereira & Vicente, 2010). Aside from electro-based processing techniques, cold plasma is novel non-thermal technology which has been applied and investigated on food samples in recent decades (Pankaj & Keener, 2018). But, in order to properly use plasma treatments, it is crucial to find adequate plasma systems that can be applied on solid or liquid food, directly or indirectly, at atmospheric or low pressure, and also at various processing parameters such as high or low frequencies and voltages. Current research of electro based non-thermal treatments of fruit juices have shown smaller microbial inactivation when compared to conventional heat treatment, and often, it is not possible to assure adequate quality and safety of food products with non-thermal treatments alone (Barba et al.,2017; Montanari et al., 2019). There is a challenge to optimize the plasma process to achieve maximum inactivation of microorganisms and to ensure mild effect on nutritionally valuable compounds. With the purpose to obtain juice quality after non-thermal processing, quick but thorough analysis must be taken. Electron paramagnetic resonance (EPR) spectrometry can be used to track free radicals in food and to observe food stability after non-thermal food techniques based on free radical production, due to its high sensitivity at low amounts of sample (Barba et al., 2020).
The aim of the present study was to investigate the effect of heat assisted plasma treatment on inactivation of *S. cerevisiae* ATCC 204508 cells in exponential growth phase in apple juice. Aside from microbial safety, quality analysis was performed regarding the physicochemical properties (pH, electrical conductivity, colour) with special accent on the antioxidant activity measured by EPR, as well as the content of phenolic compounds of apple juice.

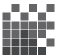
Material and methods

Sample Preparation

Apple juice was prepared by dilution from concentrated juice obtained from company Vindija d.d. (Varaždin, Croatia). Concentrated juice of 70 °Brix was diluted with water to minimum of 11.2 °Bx. Soluble solids were determined using digital refractometer (DR201-95, Kruss, Germany). *S. cerevisiae* ATCC 204508 was obtained from the Collection of Microorganisms of the Laboratory of General Microbiology and Food Microbiology, Faculty of Food Technology and Biotechnology, University of Zagreb (Zagreb, Croatia). Yeast culture was prepared by inoculating 10 ml of sterile malt extract broth (Biolife, Milan, Italy) with *S. cerevisiae* ATCC 204508 culture at 30 °C for 20 h. Harvested cells were washed three times and re-suspended in the 10 mL sterile phosphate buffer saline (PBS) and then in 10 mL sterile deionized water.

HVED plasma processing of apple juice samples

The plasma treatments were conducted in a 1000 mL vessel with a point to point electrode configuration in so called “hybrid” reactor with discharges above and in the treated volume of 200 mL of apple juice (Fig. 1). The stainless-steel ground electrode was mounted through the rubber cap and placed above the liquid, while trough the high voltage (HV) electrode stainless steel needle (Microlance TM 3.81 cm) argon (purity 99.99%; Messer Croatia, Zagreb, Croatia at the gas flow of 4 L/min) was bubbled. The plasma was generated by high-voltage (HV)



pulsed power supply (Spellman, Pulborough RH20 2RY, UK), by charging a load capacitor of 1.13 nF to up to 30 kV and then discharging the stored charge into the plasma reactor via a rotating spark gap. The voltage in the plasma reactor was measured and recorded using a Tektronix P6015A (Beaverton, OR 97077, USA) HV probe connected to a Hantek DS05202BM (Shandong, China) oscilloscope. The temperature of untreated and treated samples was monitored using an InfraRed Thermometer PCE-777 (PCE Instruments, Meschede, Germany) electrical conductivity and pH were measured using a conductivity and pH meter (HI-2030-edge, Hanna Instruments, Leighton Buzzard LU7 4AD UK).

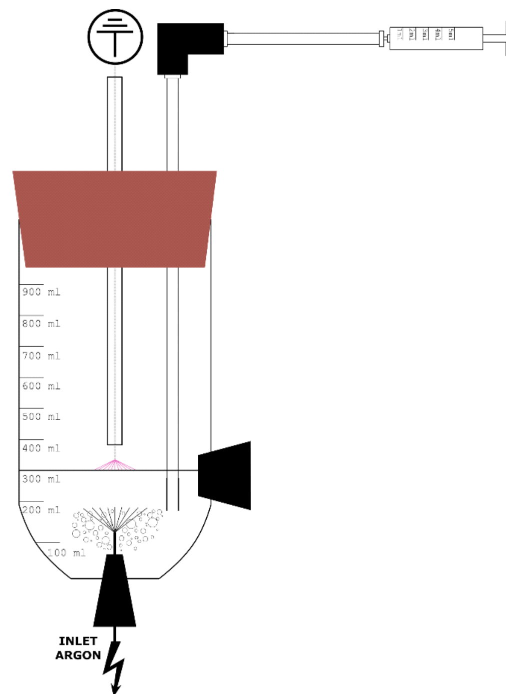


Figure 1. *Shematic description of the experimental setup: Plasma reactor*

Determination of S. cerevisiae inactivation

The total viable cell count was performed by standard dilution method on malt extract agar after incubation at 30 °C for 24 h. Ten microliter aliquots of 6-fold serial dilutions were plated on malt extract agar in a triplicate. The results were reported as log colony forming units per millilitre (log_{10} CFU/mL).

Determination of phenolic compounds

The extract of each sample was prepared in two replicates. Separation and quantification were performed using HPLC (Agilent 1260 system, Santa Clara, California) with quaternary pump, degasser, thermostated autosampler, termostated column compartment and UV/Vis-Photo Diode Array detector. Phenolic compounds were separated on Nucleosil 100-5C16, 5 µm (250 x 4.6 mm I.D.) column (Phenomenex, Los Angeles, CA). Separation was performed with slightly modified method from literature (Mitić et al., 2012).

Determination of antioxidant activity by electron paramagnetic resonance (EPR) spectrometry

The freshly prepared 0.15 mM DPPH (Sigma-Aldrich, St. Louis, USA) free radical solution in ethanol was added to the sample in order to obtain a 5% (w/v) final solution, mixed in the flask for 10 s and then put into the capillary which was then placed in a standard EPR quartz tube. (Bartoszek & Polak, 2012; Polovka et al., 2003). EPR spectra were recorded as a function of time starting from the sample and radical solution contact. EPR spectra were performed using a Varian E-109 spectrometer (Varian Instrument Division, Palo Alto, Canada) equipped with a Bruker ER041 XG microwave bridge (Bruker, Karlsruhe, Germany) operating at the X-band frequency at room temperature.

Colour measurement

The chromatic characteristics measurements of untreated and treated samples were carried out using a colorimeter CM3500d (Konica Minolta, Japan). Measurement control was performed using the SpectraMagic NX software. Chromatic variables L^*, a^* and b^* were measured in apple juice samples using CIELab method (CIE, 1976). Other chromatic variables, such as chroma (Eq. (2)), hue angle (Eq. (3)) and colour change (Eq. (4))) were calculated from:

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

$$H^\circ = \left(\frac{b^*}{a^*}\right) \qquad (3)$$

$$\Delta E^*_{ab} = \sqrt{\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2}} \qquad (4)$$

Experimental design

Experiment was designed in STATGRAPHICS Centurion (StatPoint technologies, Inc, VA 20186, USA) software. Experiment was consisted of 27 experimental trials (Table 1). The variables were treatment time (min), frequency (Hz), and temperature (°C). Analysis of variance (ANOVA) was carried out to determine any significant differences ($p<0.05$) among the applied treatments.

Results and discussion

Plasma treatment effects on yeast inactivation

In this study, S. cerevisiae in exponential phase of growth was used as a target microorganism to confirm the effectiveness of HVED plasma as microbial inactivation technique (Figure 2). Time (p=0.0002), frequency (p=0.0003), interaction of time and frequency (p=0.0099) and interaction of frequency (p=0.0090) had a significant influence on S. cerevisiae inactivation. The greatest reduction of S. cerevisiae has been noticed in samples A26 (6.6 log10 CFU/ml; after applied treatment parameters: 9 min, 120 Hz, 40 °C) and A27 (5.3 log10 CFU/ml; 9 min, 120 Hz, 50 °C) (Figure 2), which attains FDA requirements of a minimum 5 log10 reduction of spoilage and pathogenic bacteria in fruit juices. Influence of higher frequency was also observed in samples A19 and A22-25, in which were achieved reductions of 2.1 up to 3.1 log10 CFU/ml. When compared to frequency parameter, influence of time was weaker in inactivation rate since the 9 min of treatment at 60 Hz and 90 Hz achieved an inactivation of 2.2 log10 CFU/ml (A8, 9 and A17, 18).

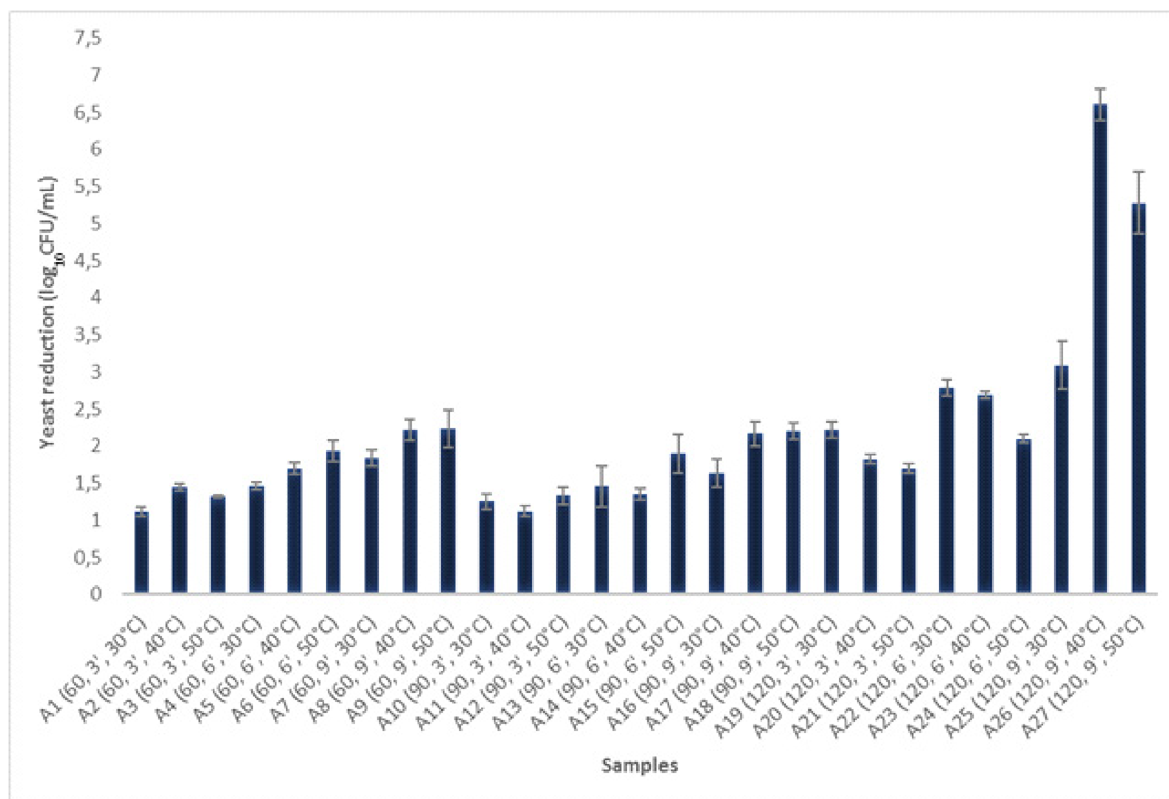
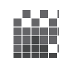


Figure 2. *Results of plasma-induced inactivation of Saccharomyces cerevisiae in apple juice.*

Effectiveness of HVED plasma in microorganism inactivation depends on microbial species, initial inoculum, and physiological phase. When compared to bacteria, yeasts are more resistant to plasma treatment due to more rigid cell walls made of chitin (Liao et al., 2017), which was also reported by Sharkey et al. (2015) who have achieved a lower reduction in S. cerevisiae cells compared to various gram positive and negative bacteria. Major components of yeast cell membrane, along with polysaccharides, are lipids and proteins which can be affected by plasma reactive species resulting in fatty acid peroxide formation and protein oxidation leading to cell death (Devi et al., 2017a). Stulić et al. (2019) studied a proteomic response of S. cerevisiae cells after HVED plasma treatment and have reported how cells activate defence mechanisms as a response to overcome stress conditions and despite lethal injuries some cells were able to recover. Inactivation mechanism of HVED is still elusive and unexplained, but there are several possible ways of microbial inactivation. Energetic electrons, produced by electrical excitation, transfer their energy to neutral gas atoms in processes that create chemically charged reactive species (Misra et al., 2016), which can accumulate on an outer cell membrane and cause its rupture (Devi et al., 2017; Yu et al., 2005). Plasma forms •OH radicals which can react with nearby molecules leading to chain oxidation and destruction of DNA (Dobrynin et al., 2009). It is very difficult to conclude which plasma components are the most effective in microbial inactivation, but the most likely it is due to a combined effect of physical, chemical, and electrical effects.

Plasma treatments are known for high radical production which influences pH changes, but it is important to mention naturally present compounds in fruit juices like vitamins, polyphenols and other bioactive compounds that are known as scavenging molecules. In apple juice, higher electrical conductivity is also measured, which influences on the plasma characteristics (shortens streamers length <1 mm), which leads to less plasma chemical interaction with surrounding compounds (Šunka, 2001). Yannam and co-authors (2018) have reported slight changes in pH of plasma treated tangerine juice. Temperature in this treatment is mostly related to heating pre-treatment and does not represent valid plasma related temperature changes. As it can be seen on Figure 1, plasma reactor was not jacketed, so decrease in temperature is due to juice cooling which is the most expressed in preheating at 50 oC (A3, A6, A9, A12, A21). Plasma heating can be seen on samples obtained at longer treatment time and lower pre-heating temp of 30 oC (A4, A7, A10, A16, A22 and A25) and 40 oC (A17 and A26). The highest increases in temperature of 6 and 8 oC were observed in samples A25 and A16, which also had high delivered energy of 7001.32 and 5467.5 J/experiment. Applied voltage, power and energy of the capacitor during and after the treatment are shown in Table 1. It is evident that the increase in frequency results in the increase of energy. Maximum energy



consumption has been reported for samples A22 (6 min, 120 Hz, 30 °C) and A23 (6 min, 120 Hz, 40 °C). Applied energy determines the energy utilization of HVED plasma treatment, and therefore, optimization of parameters is necessary to ensure the effectiveness of the treatment and quality of treated fruit juices.

Table 1. *HVED treatments: The discharge energy of the capacitor during and after the treatment*

Sample	Voltage (kV)	Power (W)	Energy per experiment (J/exp)	Energy per juice volume (J/L)
A1	28.40	18.15	3266.57	16332.84
A2	27.00	16.40	2952.45	14762.25
A3	31.00	21.62	3892.05	19460.25
A4	27.60	17.14	3085.13	15425.64
A5	36.60	30.14	5425.22	27126.09
A6	26.60	15.92	2865.62	14328.09
A7	33.00	24.50	4410.45	22052.25
A8	31.00	21.62	3892.05	19460.25
A9	32.00	23.04	4147.20	20736.00
A10	28.80	27.99	5038.85	25194.24
A11	25.80	22.47	4043.76	20218.82
A12	30.00	30.38	5467.50	27337.50
A13	30.00	30.38	5467.50	27337.50
A14	27.40	25.34	4560.87	22804.34
A15	27.60	25.71	4627.69	23138.46
A16	30.00	30.38	5467.50	27337.50
A17	29.00	28.38	5109.08	25545.38
A18	23.60	18.80	3383.53	16917.66
A19	25.00	28.13	5062.50	25312.50
A20	26.00	30.42	5475.60	27378.00
A21	21.60	21.00	3779.14	18895.68
A22	30.00	40.50	7290.00	36450.00
A23	30.00	40.50	7290.00	36450.00
A24	25.00	28.13	5062.50	25312.50
A25	29.40	38.90	7001.32	35006.58
A26	24.60	27.23	4901.80	24508.98
A27	26.60	31.84	5731.24	28656.18

Effect on the stability of phenolic compounds

Results of the quantitative analysis of individual phenolic compounds in untreated (A0) and treated samples (A1-A27) with HVED plasma are presented in Table 1. It can be observed that there was not a significant influence neither of treatment time (p=0.67-0.78), frequency (p=0.006-0.14) or temperature (p=0.17-0.95) on individual phenolic compounds. Optimum value for all observed phenolic compounds was obtained with the following treatment: treatment time = 6 min; temperature = 50 °C; frequency = 60 Hz. Hydrophilic compounds, such as chlorogenic acid, are found in higher concentration in apple juice than hydrophobic compounds, such as quercetin (Massini et al., 2018), which is in accordance with the results of this study. Most of quercetin is present in glycosylated form with glucose, galactose, rhamnose, xylose and arabinose (Petersen et al., 2016). Total phenolic content (TPC) was calculated as sum of the contents of all individual phenolic compounds. HVED plasma treatment, in almost all samples, caused an increase in TPC (Table 1). The highest TPC was recorded for samples which were treated at frequency of 60 Hz and the maximum is determined in sample A9 (9 min, 60 Hz, 50 °C). Herceg and co-authors (2016) also reported an increase in TPC in pomegranate juice after gas phase plasma treatment as well as Elez Garofulić and co-authors (2015) in sour cherry Marasca juice an increase in anthocyanin and phenolic acid content. Decreasing in TPC was determined for all samples treated at frequency of 120 Hz through 9 min (A25, A26 and A27). Grzegorzewski et al.(2011) also reported decreasing of TPC in lettuce leaves after exposure to an atmospheric pressure plasma jet as well as Pankaj and Keener (2017) in white grape juice after HVED plasma treatment. Degradation of phenolic compounds can be explained by their ability to scavenge free radicals generated by cold plasma, which leads to cleavage of the central heterocycle in polyphenolic skeleton (Makris & Rossiter, 2002) and oligomerization. These different results can be explained by different phenolic content in fruit juices. Phenolic compounds differ in chemical composition and thus in antioxidative properties, which manifest in the

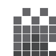
interaction with plasma reactive species (Grzegorzewski et al., 2011). Plasma produces free radicals, shock waves, light emissions, and cavitation, which all affect phenolic compounds (Locke et al., 2006). Therefore, plasma parameters should not be neglected. Elez Garofulić et al. (2015) reported that treatment time and sample volume have been major factors influencing the increase in phenolic compounds. Plasma influence on phenolic compounds in fruit juices is not completely clear and further investigations are necessary.

Table 2. *Average concentrations (mg L^{-1}) of individual and total (TPC) phenolic compounds in untreated and plasma treated samples and antioxidant activity (%).*

Sample	Phloridzin			Chlorogenic acid			p-coumaric acid derivates			Quercetin			TPC			Antioxidant activity (%)		
A0	3.11	±	2.97	12.63	±	3.08	0.72	±	0.11	0.79	±	0.10	17.25	±	6.26	47.92	±	5.59
A1	3.49	±	1.48	15.33	±	0.65	0.87	±	0.04	0.87	±	0.04	20.56	±	2.22	65.98	±	6.83
A2	3.54	±	0.92	15.96	±	0.18	0.89	±	0.02	0.88	±	0.03	21.27	±	1.14	63.26	±	5.17
A3	3.91	±	0.07	17.44	±	0.46	0.90	±	0.02	0.95	±	0.02	23.21	±	0.56	69.17	±	9.16
A4	3.61	±	0.49	14.81	±	0.77	0.85	±	0.02	0.89	±	0.03	20.16	±	1.31	63.93	±	6.81
A5	3.53	±	0.85	16.11	±	0.88	0.90	±	0.02	0.89	±	0.03	21.43	±	1.77	50.18	±	4.31
A6	3.35	±	3.96	17.18	±	1.04	1.14	±	0.32	0.76	±	0.25	22.43	±	5.58	54.2	±	6.58
A7	3.57	±	0.35	14.78	±	0.02	0.84	±	0.01	0.88	±	0.01	20.06	±	0.39	53.55	±	3.86
A8	3.32	±	1.41	15.93	±	0.40	0.85	±	0.06	0.83	±	0.05	20.93	±	1.92	56.5	±	9.48
A9	3.81	±	0.49	17.96	±	0.18	0.92	±	0.01	0.93	±	0.01	23.62	±	0.70	70.03	±	4.92
A10	3.61	±	1.91	17.25	±	1.37	0.91	±	0.06	0.9	±	0.05	22.66	±	3.39	60.01	±	6.72
A11	3.71	±	0.00	16.44	±	0.95	0.90	±	0.03	0.92	±	0.03	21.98	±	1.00	46.16	±	6.98
A12	3.29	±	1.48	14.95	±	0.62	0.85	±	0.03	0.85	±	0.04	19.94	±	2.18	56.47	±	6.14
A13	3.19	±	1.91	14.8	±	1.54	0.82	±	0.06	0.80	±	0.08	19.61	±	3.58	57.89	±	4.24
A14	3.39	±	1.56	15.82	±	1.34	0.88	±	0.05	0.85	±	0.05	20.95	±	2.99	63.77	±	8.76
A15	3.66	±	0.99	14.62	±	0.35	0.84	±	0.01	0.91	±	0.02	20.03	±	1.37	64.96	±	8.02
A16	3.46	±	0.57	15.23	±	0.40	0.88	±	0.02	0.86	±	0.02	20.43	±	1.01	55.07	±	7.64
A17	3.51	±	1.84	15.38	±	0.87	0.86	±	0.06	0.89	±	0.07	20.65	±	2.83	47.84	±	7.47
A18	3.25	±	0.42	14.88	±	0.17	0.84	±	0.00	0.83	±	0.00	19.80	±	0.59	50.75	±	6.05
A19	3.45	±	2.69	14.29	±	1.58	0.72	±	0.09	0.88	±	0.08	19.34	±	4.44	53.66	±	1.71
A20	3.30	±	3.39	12.36	±	1.40	0.73	±	0.06	0.83	±	0.10	17.23	±	4.95	65.65	±	7.73
A21	3.20	±	2.83	12.64	±	1.76	0.67	±	0.05	0.81	±	0.07	17.31	±	4.71	63.26	±	5.61
A22	3.34	±	1.48	14.41	±	1.47	0.73	±	0.07	0.84	±	0.05	19.32	±	3.07	52.02	±	9.49
A23	3.39	±	1.06	14.19	±	0.43	0.66	±	0.10	0.87	±	0.01	19.11	±	1.60	58.55	±	10.37
A24	3.37	±	0.57	12.50	±	0.53	0.71	±	0.00	0.84	±	0.03	17.42	±	1.12	48.45	±	4.18
A25	3.29	±	4.88	8.87	±	1.58	0.68	±	0.13	0.81	±	0.14	13.66	±	6.73	53.28	±	4.59
A26	3.19	±	3.61	12.00	±	2.32	0.69	±	0.11	0.81	±	0.10	16.70	±	6.14	53.31	±	11.09
A27	3.37	±	0.57	12.50	±	0.53	0.71	±	0.00	0.84	±	0.03	17.42	±	1.12	46.28	±	5.09

Evaluation of antioxidant activity

EPR method has already been used in plasma treated liquids (Jablonowski et al., 2015; Tresp et al., 2013), but the scarce reports can be found for plasma treated real foods (Wang et al., 2012). Results of the evaluation of antioxidant activity using EPR method in untreated (A0) and treated samples (A1-A27) with HVED plasma are presented in Table 2. There was not a significant influence neither of treatment time (p=0.08), frequency (p=0.11) or temperature (p=0.79) on antioxidant activity. However, it can be seen that the highest antioxidant activity was obtained for samples which were treated at frequency of 60 Hz (A1-4) and the maximum is determined in sample A9 (, 9 min, 60 Hz , 50 °C), the one with the highest TPC. Oszmianski et al.,(2007) have compared clear and cloudy apple juice and have concluded that the juice with

the highest TPC resulted in the highest antioxidant activity. In samples treated at 90 Hz for 6 min (A14, A15) and even 120 Hz for 3 min (A20, A21) DPPH radical scavenging increased for 15-17.73%. Zhang et al. (2015) reported that pulsed electric field treatment of vitamin C caused DPPH radical scavenging activity to stay stable or to increase. DPPH radical scavenging activity was unchanged, or it slightly decreased in some samples (A11, A17, A 24 and A27). This can be expected, as TPC gives a major contribution to very good antioxidant properties of apple juices. By using EPR method it is possible to detect undesirable changes in treated foods by identifying the nature of free radicals generated by cold plasma treatment (Barba et al., 2020). Furthermore, no significant influence neither of treatment time, frequency, or temperature ($p>0.05$) on antioxidant activity, gives a good promise that the applied treatment retains original properties of monitored samples.

Effect on colour

Juice colour plays a great role in food choice of consumers. The effect of applied plasma treatment on the colour of apple juice is presented in Table 3. Values of variable L* ranged from 99.98 (sample A22 – 6 min, 120 Hz , 30 °C) to 98.06 (sample A9 – 9 min, 60 Hz, 50 °C) in treated samples (Table 3), while L* value in untreated sample was 99.92. The results indicate that plasma treatment has not caused significantly browning of apple juice. Temperature (L* p=0.04; b* p=0.03), frequency (L* p=0.04), and interaction of temperature and frequency (L* p=0,03, b* p=0.00) had a significant influence on colorimetric variables L* and b*, while the variable a* was not influenced by neither of treatment time (p=0.94), frequency (p=0.77) or temperature (p=0.77).

In the same samples that were heated to 50 °C before plasma treatment, brighter colour was noticed, which can be explained with protein denaturation or with deactivation of enzymatic oxidation at higher temperatures (Massini et al., 2018). Optimum value for the parameter L* (99.92) was obtained with the following treatment: time = 3.01 min; temperature = 30.04 °C. Lukić et al. (2019) reported more significant decrease of variable L* in red wine (from 26.28 to 24.51) during HVED treatment in hybrid reactor with increasing of treatment time and frequency as well as Bursać Kovačević et al. (2016) in pomegranate juice (from 23.51 to 20.42) after cold atmospheric gas phase plasma treatment. Opposite to that, Almeida et al. (2015) reported that exposition of prebiotic orange juice to plasma field at 70 kV caused slightly increase of variable L* by about 3 units in prebiotic orange juice after 15, 30 and 60 sec of treatment. The analysed apple juice is expected to position in the green-yellow region of the chromatic space, which is in accordance with experimental data. Significant increase of variable b* was noticed in samples which were heated at 50 °C before plasma treatment and then treated at frequency of 60 Hz. As shown in Table 2, decrease of H° values in all treated samples after plasma treatment was observed and ranged from 87.38 ° (A3 – 3 min, 60 Hz, 50 °C) to 110.21° (A17 – 9 min, 90 Hz, 40 °C) (Table 3), which indicates that apple juice was becoming less yellow-green. A significant increase of variable C* was noticed in samples A3 (3 min, 60 Hz, 50 °C), A9 (9 min, 60 Hz, 50 °C) and A6 (6 min, 60 Hz, 50 °C) (Table 3, which means that plasma treatment in these samples resulted in more vivid instrumental colour. Bursać Kovačević et al. (2016) reported an increasing of variable L* with the higher sample volume and increasing of variables L*, a*, b* and C* with the increase of gas flow. Calculated total colour difference (ΔE) for almost all samples has been in “slightly noticeable” range ($0.5<\Delta E<1.5$). The highest colorimetric difference was determined in sample A26 (ΔE = 2; 9 min, 120 Hz, 40 °C) and the lowest in sample A23 (ΔE = 0.34; 6 min, 120 Hz, 40 °C) (Table 3). “Well visible” changes ($3<\Delta E<6$) have not been noticed in none of the treated samples, so it can be concluded that the characteristic colour of the juice was kept in the expected range.

Table 3. *Colour parameters for untreated and treated samples.*

Sample	L*			a*			b*			C*			Ho			ΔE		
A0	99.92	±	0.01	-0.16	±	0.02	0.60	±	0.05	0.62	±	0.00	105.14	±	0.16	/	±	
A1	99.91	±	0.04	-0.13	±	0.02	0.57	±	0.09	0.58	±	0.00	100.48	±	0.08	0.78	±	0.00
A2	99.90	±	0.02	-0.12	±	0.01	0.74	±	0.03	0.75	±	0.08	103.02	±	0.16	0.36	±	0.02
A3	98.18	±	0.02	0.06	±	0.01	1.34	±	0.94	1.34	±	0.37	87.38	±	0.77	1.41	±	0.01
A4	99.95	±	0.01	-0.09	±	0.01	0.53	±	0.71	0.54	±	0.03	100.16	±	0.02	1.01	±	0.14
A5	99.80	±	0.32	-0.14	±	0.01	0.38	±	0.02	0.40	±	0.01	102.39	±	0.02	0.68	±	0.02
A6	99.97	±	0.41	-0.20	±	0.1	1.52	±	0.02	1.53	±	0.02	102.80	±	0.01	1.50	±	0.01
A7	99.65	±	0.21	-0.09	±	0.02	0.72	±	0.07	0.73	±	0.02	97.05	±	0.1	0.92	±	0.01
A8	99.62	±	0.1	-0.05	±	0.05	0.67	±	0.06	0.67	±	0.05	94.06	±	0.45	0.46	±	0.01
A9	98.06	±	0.02	0.05	±	0.04	1.18	±	0.03	1.18	±	0.11	87.45	±	0.063	0.66	±	0.01
A10	99.96	±	0.02	-0.10	±	0.01	0.53	±	0.03	0.54	±	0.01	99.76	±	0.07	1.17	±	0.03
A11	99.93	±	0.03	0.00	±	0.12	0.74	±	0.01	0.74	±	0.01	97.35	±	0.01	1.97	±	0.04
A12	99.86	±	0.01	-0.12	±	0.01	0.58	±	0.01	0.59	±	0.02	99.87	±	0.05	1.76	±	0.01
A13	99.95	±	0.1	-0.11	±	0.13	0.50	±	0.01	0.51	±	0.21	101.97	±	0.01	1.48	±	0.11
A14	99.77	±	0.21	-0.10	±	0.04	0.56	±	0.11	0.57	±	0.01	100.01	±	0.01	0.89	±	0.01
A15	99.69	±	0.3	-0.10	±	0.07	0.54	±	0.01	0.55	±	0.04	92.77	±	0.01	0.99	±	0.03
A16	99.78	±	0.4	-0.11	±	0.09	0.64	±	0.52	0.65	±	0.06	99.96	±	0.9	1.19	±	0.02
A17	99.95	±	0.31	-0.11	±	0.16	0.48	±	0.04	0.49	±	0.01	110.21	±	0.02	1.58	±	0.02
A18	99.71	±	0.12	-0.08	±	0.01	0.60	±	0.71	0.61	±	0.02	90.26	±	0.02	1.29	±	0.05
A19	99.78	±	0.15	-0.04	±	0.1	0.74	±	0.09	0.74	±	0.01	94.85	±	0.05	1.32	±	0.06

A20	99.89	±	0.17	-0.14	±	0.02	0.25	±	0.06	0.29	±	0.03	101.73	±	0.04	1.52	±	0.06
A21	99.92	±	0.01	-0.11	±	0.2	0.50	±	0.08	0.51	±	0.1	103.40	±	0.04	1.49	±	0.01
A22	99.98	±	0.01	-0.08	±	0.1	0.62	±	0.01	0.63	±	0.21	97.16	±	0.09	0.97	±	0.01
A23	99.76	±	0.04	-0.01	±	0.02	0.75	±	0.06	0.75	±	0.37	90.42	±	0.05	0.34	±	0.37
A24	99.91	±	0.01	-0.12	±	0.03	0.52	±	0.01	0.53	±	0.06	103.13	±	0.12	0.34	±	0.12
A25	99.93	±	0.01	-0.07	±	0.01	0.52	±	0.01	0.52	±	0.12	99.06	±	0.015	1.02	±	0.01
A26	99.67	±	0.02	-0.10	±	0.08	0.56	±	0.01	0.57	±	0.15	97.37	±	0.4	2.00	±	0.01
A27	99.90	±	0.04	-0.12	±	0.01	0.56	±	0.01	0.57	±	0.03	97.89	±	0.001	1.38	±	0.02

Conclusions

Microbial safety, colour stability and nutritional value were the main goals to evaluate a possible application of HVED plasma treatment on fruit juices. Combination of pre-heating with HVED treatment assured significant reduction of *S. cerevisiae* in 9 min with the pre-heating temperature of 40 °C and applied frequency of 120 Hz. Lower frequency and shorter treatment time were the most effective for the colour and phenolic stability as well as antioxidant activity. Applied HVED treatments have shown its applicability in maintaining microbial safety and ensuring high quality parameters of apple fruit juice. For future food preservation by plasma devices is fundamental to clarify are the yeast cells really inactivated or only injured with the possibility to recover. This could not only present a health risk, but also could raise questions when and for what type of food is plasma treatment applicable in terms of food preservation. However, this process requires further investigations on other types of juices as well as other liquid food samples.

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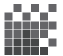

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