applied sciences

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Quality of Tomato Juice as Influenced by Non-Thermal Air Plasma Treatment

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Abstract: This paper presents the results of an experiment based on using a Glide-arc type plasma reactor operating at atmospheric pressure for the quality of fresh pressed tomato juice, variety Bekas. The impact of after-glow plasma gas (air) on the physicochemical, microbiological properties and morphology of the product's samples was investigated. Five groups of juices characterized by different exposure times (30, 60, 120, 300 and 600 s), as well as untreated juice (as control) were used. The juice quality was assessed on days 1, 3, 5, and 10 of refrigerated storage. Significant increases were observed when Cold Atmospheric Plasma (CAP)-treated tomato juice was tested against total soluble solids, pH, lycopene, and vitamin C in comparison to the control treatments. Moreover, changes in the tested physicochemical values during the storage of juice subjected to the action of cold plasma did not progress as quickly as in the case of the control juice. A significant decrease was observed in total plate count, yeast, and mold after 300–600 s CAP treatment. The findings of the current study suggested that CAP treatment is a promising technique that could provide improved quality and stability during the processing of tomato juice with better physicochemical properties and bioavailable nutrients.

Keywords: atmospheric pressure plasma; Glide-arc reactor; microbial bio-decontamination; physico-chemical and morphological properties; fresh pressed tomato juice; electrotechnology for food

Citation: Starek-Wójcicka, A.; Sagan, A.; Terebun, P.; Kwiatkowski, M.; Osmólska, E.; Krajewska, M.; Grządka, E.; Matsuyama, N.; Hayashi, N.; Pawlat, J. Quality of Tomato Juice as Influenced by Non-Thermal Air Plasma Treatment. *Appl. Sci.* **2023**, *13*, 578. https://doi.org/10.3390/app13010578

Academic Editor: Suyong Lee

Received: 13 December 2022
Revised: 23 December 2022
Accepted: 27 December 2022
Published: 31 December 2022

1. Introduction

Currently, consumers are eager to buy products that exert a positive effect on their health and wellbeing and protect their bodies against diseases. A healthy human diet should contain large amounts of raw or processed fruit and vegetables. NFC (Not From Concentrate) juices are part of the currently promoted so-called healthy lifestyle. They are made only from fresh raw material without the addition of water, concentrated juice, or artificial ingredients; hence, they are definitely more valuable than 'From Concentrate' juices. They provide the organism with substantially higher amounts of vitamins, minerals, dietary fiber, pectins, and many substances that are essential for health, e.g., antioxidants (flavonoids or carotenoids) [1–3]. Most of these juices are pasteurized to eliminate microorganisms present in the products. Elimination of bacteria protects the products against spoilage, thereby extending their storage stability over a long time. However, this treatment

process can destroy not only microorganisms but also many valuable biologically active substances [4–6].

Therefore, there is a need to devise methods for juice preservation that will induce the smallest possible changes in the physicochemical properties of the material without compromising its microbiological safety. The application of cold atmospheric plasma (CAP), classified as a low-temperature treatment contributing to the preservation of thermolabile substances in juice, seems to be a promising approach. A properly designed experimental plasma system and properly selected process parameters can yield very high-quality products. Cold plasma has become a novel option as a disinfecting and preserving agent, which can also improve the quality of certain food parameters [7–9]. Many research groups reported that relatively low temperatures allowed the treatment of a variety of samples, including biological material, such as seeds and food, and including juices and nectars [10–16]. Plasma is generated in electrical discharges, and depending on the operational parameters of the device, and many factors, such as free electrons and electromagnetic radiation, species such as RONS (reactive oxygen and nitrogen species) can be generated within plasmatrons and transported to the sample via working gas [17–20].

The aim of the current work was to investigate the impact of low temperature plasma on tomato juice. Air, as the most affordable option, was selected as a substrate gas.

2. Materials and Methods

2.1. Juice Preparation

Fresh red tomatoes vs. Bekas harvested from eastern Poland were classified, washed, and pressed to make juice by using a Philips slow juicer (Model HR1889/70, Amsterdam, Holland). A portion of the juice was treated with cold plasma for a specified period of time, while the untreated drink was the control. The pH, total soluble solids (Brix), lycopene, ascorbic acid, color (L*, a*, b* and ΔE), and microbial activity were analyzed. The juice was refrigerated at 6 °C for 10 days.

2.2. Cold Plasma Treatment

Two-electrode atmospheric pressure GlidArc reactor (GAD) with AC power supply (50 Hz discharge frequency, 3.8 kV of applied voltage, 40 W of mean power) was used [17].

An amount of 440 L/h of air was supplied to the discharge zone via flow controller, and 50 mL of juice in a sterile glass container was placed on the magnetic stirrer AREX (VELP Scientifica, Usmate, Italy). The distance between the electrodes and the juice surface was 10 mm, and stirring speed was set at 120 RPM. Schema of the experimental set-up is presented in Figure 1. Juice samples were treated with plasma for 0 (control), 30, 60, 120, 300, 600 s. The highest temperature of the sample, measured after 600 s treatment with K-type thermocouple connected to the DT-847U meter (Maxtech, Taipei, Taiwan) was 29.6 °C.

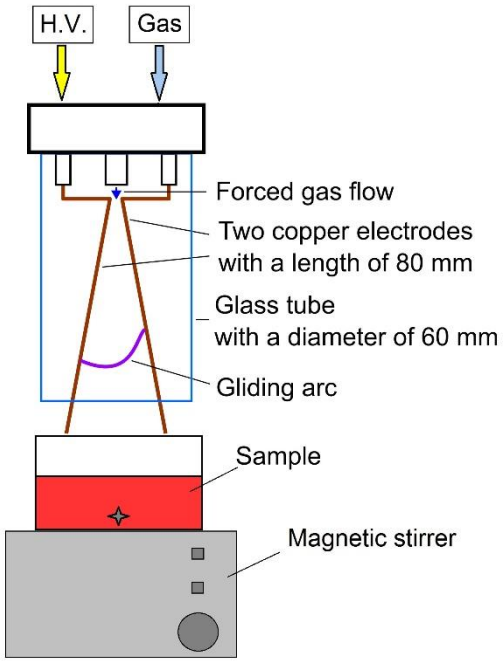

Figure 1. Experimental set-up.

2.3. Determination of Total Soluble Solids and pH

Total soluble solids (°Brix) and pH were measured using a handheld refractometer (LLG-uniREFRACTO, Meckenheim, Germany), and a pH meter (780 pH Meter Metrohm, Herisau, Switzerland) calibrated at 4.0 and 7.0 pH with standard buffers, respectively. The analysis was performed at 25 ± 1 °C.

2.4. Determination of Lycopene

The lycopene content was determined using the spectrophotometric method extracting the compound with a mixture of acetone with 0.2% BHT, ethanol and hexane (1: 1: 2) from the tested sample. Absorbance was measured at 503 nm using a UV/Vis Helios Omega 3 spectrophotometer (Massachusetts, MA, USA).

2.5. Determination of Ascorbic Acid

The content of vitamin C (L-ascorbic acid) was determined using 2,6-dichlorophenoloindophenol (Tillmans dye) in accordance with Hallmann (2012). The sample was extracted in 2% oxalic acid. The solution was filtered. The filtrate was collected and then titrated with the Tillmans dye until a permanent pink color was reached.

2.6. Determination of Color Parameters in Tomatoes Juice

The color of the tomato juices was measured using a 3Color spectrophotometer SF80 (Marcq-en-Barœul, France). The results were expressed in accordance with the CIE L*a*b* system, using illuminant D65 and 10° observer. The color parameters were L* value (lightness, ranging from 0, black, to 100, white), a* value (positive values for reddish colors and negative value for greenish ones), and b* value (positive for yellowish colors and negative for bluish ones). A standard white plate (L* = 92.37, a* = −0.82 and b* = 1.82) was used to calibrate the instrument. Moreover, the tomato juice was critically evaluated for the total color difference (ΔE), which showed a numerical difference in this parameter of the samples after CAP treatment compared to the control sample, according to Equation (1).

$$\Delta E = \sqrt{(L^* - L_0)^2 + (a^* - a_0)^2 + (b^* - b_0)^2} \quad (1)$$

where, L_o, a_o, and b_o are the color values of untreated juice samples.

The following criterion was used in the analysis of the results: the absolute color differences (ΔE) between 0 and 1 were regarded as unrecognizable (indiscernible deviation), values from 1 to 2 denoted a slight deviation recognizable by a person experienced in discriminating color nuances, values in the range of 2–3.5 indicated a deviation recognized even by a non-expert, 3.5–5 represented a clear deviation, and ΔE above 5 denoted a substantial color deviation.

2.7. Microbiological Analysis

The total numbers of aerobic microorganisms of treated and control tomato juice samples were carried out by following PN-EN ISO 4833–2, and the number of yeasts and molds as recommended by the international standard PN-ISO 21527–2, respectively. The microorganisms present in test samples were diluted and applied to the center of a previously prepared agar plate. Procedures were performed in sterile conditions—a laminar chamber with a CRUMA 670FL UV lamp (El Prat de Llobregat, Barcelona, Spain). Incubation was carried out in a POL-EKO type CLN 115 SMART (Wodzislaw Ślaski, Poland) incubator at 30 °C for 72 h for microorganisms on PCA medium (agar with casein, glucose, yeast extract hydrolyzate) and at 25 °C for 5 days for yeasts and molds on a medium containing agar with a mixture of peptones, glucose, and chloramphenicol. Bacterial colonies were counted in the juice samples as CFU/mL of tomato juice and expressed as log CFU/mL of the sample.

2.8. Microscopic Analysis

The control and plasma-treated samples were observed using VK-X1000 laser microscope (Keyence Int., Osaka, Japan), which combines white light with a laser light source. The droplet of juice was put onto the microscopic glass. Non-contact profile and 3D depth analysis were performed to observe the microstructure of the juice and microscopic photos of the samples were taken.

2.9. Statistical Analysis

The individual physicochemical properties were analyzed in triplicate and presented as mean values with standard deviation (SD). In turn, the microbiological analysis results were presented as the mean values of four inoculations of each sample together with standard deviation (SD).

Additionally, the Tukey test (Statistica 10, StatSoft Inc., Tulsa, OK, USA) was used to determine the effect of the factors on the physicochemical properties of the tomato juice. Particular attention was paid to the comparison of the control group with the individual levels of factors on the analyzed storage days (1, 3, 5, and 10). It was equally important to determine the effect of the cold plasma treatment time (30, 60, 120, 300, and 600 s) on changes in the analyzed parameters in comparison with the unprocessed samples.

3. Results and Discussion

3.1. Effect of CAP on Physicochemical Properties

Plasma technology uses plasma active species including radicals, neutral species, UV photons, and charged particles; therefore, interaction with some food components is possible. Evaluation of physicochemical properties after processing of the food material would help to reveal the effect of plasma, since any change may indicate a possible chemical reaction with one or more food components.

Acidity is one of the key parameters of juice quality, as it has an impact on the preservation method used for the maintenance of product safety. Moreover, any changes in this parameter may have an adverse effect on the texture, taste, and shelf life of products [21]. Tomatoes are acidic foods; hence, pH value of the tomato juices determined in the present study ranged from 3.35 to 4.57. The pH value in the control sample decreased during storage. The active acidity in the cold-plasma-treated juices was unchanged or increased slightly with the storage time. A statistically significant increase in pH value was found in

the cold-plasma-treated juice samples in comparison with the control. On the first storage day, pH of the juice exposed to cold plasma for 600 s slightly increased to 4.45, compared to 3.89 in the control sample (Table 1).

Table 1. Effect of cold plasma treatment on the chemical properties of tomato juice stored for 10 days.

		Storage Time (Days)			
Chemical Parameters	**Time of Plasma Treatment (s)**	**1**	**3**	**5**	**10**
pH	0 (control)	3.89 ± 0.02^{Ac}	3.65 ± 0.06^{Ab}	3.35 ± 0.05^{Aa}	NP
	30	4.41 ± 0.00^{Ba}	4.41 ± 0.00^{Ba}	4.42 ± 0.00^{Ba}	4.43 ± 0.00^{Aa}
	60	4.42 ± 0.01^{Ba}	4.43 ± 0.01^{Ba}	4.43 ± 0.00^{Ba}	4.44 ± 0.00^{Aa}
	120	4.46 ± 0.00^{Ba}	4.46 ± 0.00^{Ba}	$4.48 \pm 0.00B^{Ca}$	4.48 ± 0.00^{Aa}
	300	4.46 ± 0.00^{Ba}	4.55 ± 0.00^{Cb}	4.54 ± 0.03^{Cb}	4.55 ± 0.01^{Bb}
	600	4.45 ± 0.00^{Ba}	4.53 ± 0.04^{Cb}	4.52 ± 0.01^{Cb}	4.57 ± 0.02^{Bb}
Total Soluble Solids (°Brix)	0 (control)	3.67 ± 0.06^{Aa}	3.57 ± 0.06^{Aa}	3.33 ± 0.06^{Ab}	NP
	30	3.67 ± 0.06^{Aa}	3.70 ± 0.00^{Aa}	3.70 ± 0.00^{Ba}	4.10 ± 0.00^{Bb}
	60	3.70 ± 0.00^{Aa}	3.70 ± 0.00^{Aa}	3.87 ± 0.12^{Bb}	4.00 ± 0.10^{Bb}
	120	3.67 ± 0.06^{Aa}	3.67 ± 0.06^{Aa}	3.83 ± 0.06^{BCab}	3.87 ± 0.06^{Ab}
	300	3.80 ± 0.00^{Aab}	3.70 ± 0.00^{Aa}	3.93 ± 0.06^{BCb}	3.83 ± 0.06^{Aab}
	600	3.83 ± 0.06^{Aab}	3.87 ± 0.06^{Bab}	3.97 ± 0.15^{Cb}	3.77 ± 0.06^{Aa}
Lycopene (mg/100 g)	0 (control)	66.70 ± 1.20^{Ab}	65.00 ± 0.23^{Ab}	62.38 ± 0.24^{Aa}	NP
	30	67.37 ± 0.14^{Aa}	67.36 ± 0.14^{Aa}	67.37 ± 0.71^{Ba}	65.96 ± 0.14^{Aa}
	60	66.48 ± 0.78^{Aa}	65.15 ± 0.49^{Aa}	66.64 ± 0.42^{Ba}	65.35 ± 0.49^{Aa}
	120	65.20 ± 0.85^{Aa}	64.79 ± 0.71^{Aa}	64.19 ± 0.57^{Aa}	64.86 ± 0.57^{Aa}
	300	66.09 ± 0.21^{Aa}	66.09 ± 1.13^{Aa}	66.51 ± 0.49^{Ba}	66.43 ± 0.07^{Aa}
	600	66.50 ± 0.00^{Aa}	65.86 ± 0.07^{Aa}	66.74 ± 0.49^{Ba}	66.43 ± 0.04^{Aa}
Ascorbic acid (mg/100 g)	0 (control)	277.45 ± 0.49^{Bc}	261.90 ± 0.71^{Ab}	238.55 ± 0.64^{Aa}	NP
	30	275.60 ± 0.28^{Ac}	273.15 ± 0.92^{BCbc}	271.40 ± 0.14^{Bb}	266.40 ± 0.42^{Aa}
	60	273.55 ± 0.64^{Ab}	272.20 ± 2.12^{BCb}	271.80 ± 0.28^{Bb}	266.60 ± 0.28^{Aa}
	120	274.35 ± 0.64^{Ab}	271.20 ± 0.14^{Ba}	273.75 ± 0.92^{BCb}	268.30 ± 0.85^{Aa}
	300	273.30 ± 1.27^{Aa}	272.35 ± 0.49^{BCa}	273.25 ± 0.21^{BCa}	270.05 ± 0.07^{Ba}
	600	273.80 ± 2.12^{Ab}	275.05 ± 0.92^{Cb}	276.05 ± 0.21^{Cb}	268.55 ± 0.78^{ABa}

The results are expressed as a mean ± standard error. $^{a, b, c}$—statistically significant differences between the means in the columns ($p < 0.05$). $^{A, B, C}$—statistically significant differences between the means in the rows ($p < 0.05$). NP = designation not performed.

The decrease in the acidity of the cold-plasma-treated products can be explained by the formation of oxidized compounds as a result of the collision of energetic electrons with the molecular oxygen and nitrogen present in the working gas. This results in an increase in pH value; however, research in [21–23] found no changes in pH value in cold-plasma-treated grape and tomato juices. The effect of this type of treatment on pH value of food is often modified by several factors, e.g., the process parameters, the buffering capacity of the processed material, and the physiological functions of the living tissue [21].

Brix degrees were used to express the content of Total Soluble Solids (TSS). The content of TSS in the tested juices ranged from 3.33 °Brix in the control sample on experiment day 5 to 3.87 °Brix in juice exposed to 10 min treatment on day 3. The TSS value was slightly higher in the cold-plasma-treated juices on storage days 1–5, with a statistically significant increase in this parameter in all cold-plasma-treated samples versus the control sample noted on day 5 of the experiment. At that time point, the TSS value in the 600 s plasma treatment variant was almost 20% higher than in the control sample. After ten days of storage, the TSS content was higher in the juices exposed to the shorter (30 and 60 s) plasma

treatments than in the longer (120, 300, and 600 s) exposure variants. The increase in TSS can be explained by water loss induced by the cold plasma treatment of the juices (Table 1).

The human body has many defense mechanisms that neutralize the harmful effects of reactive oxygen species. Carotenoids play an important role in reducing oxidative damage. Among them, the main pigment responsible for the characteristic deep red color of ripe tomato fruits is lycopene, which is not synthesized by the human body and should be supplied with food. This compound is absorbed to the greatest extent from preserves, including juices. Lycopene in improperly processed tomato products can be quite unstable. The main causes of lycopene destruction at higher temperatures are isomerization and oxidation. Additionally, light, temperature, and storage time may change the impact rates of these two processes on lycopene content in tomato products. However, mild thermal treatment causes increased solubility and better absorption of lycopene without lowering its antioxidant potential. A technology, which meets the requirements of non-thermal methods and is of interest to the food industry is the low-temperature (cold) plasma treatment. The present study has shown lycopene content of 66.70 and 65.00 mg/100 g on days 1 and 3 of refrigerated storage of the tomato juice, respectively. These values were not statistically different ($p < 0.05$) from those obtained for the juice treated with cold plasma for 30 to 600 s. In turn, the lycopene content in the control juice on experiment day 5 decreased by approximately 6%, compared with the value recorded on day 1. The cold-plasma-treated tomato juices were characterized by unchanged content of this valuable pigment throughout the storage period (Table 1). Some previous reports also demonstrated that the lycopene content increased significantly in samples subjected to High-Voltage Electric Field Cold Plasma (HVCP) using Dielectric Barrier Discharge (DBD) treatments or to the combined effect of HVCP and Ultra-Sonication (US), which resulted in better retention of lycopene compared to fresh untreated carrot juice samples [24,25]. In a study conducted by Paixão et al. [26], seriguela juice was subjected to the plasma treatment using a glow discharge plasma generator (with nitrogen as the process gas). The lycopene content was found to increase in most of the experimental assays. Furthermore, the prominent increase in the content of carotenoid pigments is also in agreement with previous studies, in which the effect of CAP was observed in the acerola juice [27]. The better retention of lycopene during processing is mainly related to the conversion of the trans-isomer to the more bioavailable form (cis-isomer) [28].

Tomato juice contains vitamin C, which has the ability to deactivate peroxide radicals generated both during the preparation of food and as a result of metabolic processes in the organism. The content of ascorbic acid in the analyzed tomato juices ranged from 266.40 mg/100 g to 277.45 mg/100 g. The greatest losses of this compound during storage were recorded in the control sample. After five days, the content of vitamin C was approximately 17% lower than in the juice sample analyzed on day 1. These losses in the cold-plasma-treated juices were substantially lower (approximately 2–3% between days 1 and 10 of the experiment).

Ascorbic acid was not significantly destroyed by the cold plasma effect on the tomato juice; however, the content of vitamin C on the first day of storage was statistically significantly lower in the cold-plasma-treated juices than in the control sample. However, these were small differences (up to 2%). Wang et al. [29] examined the physicochemical properties of cold-plasma-treated fruits and vegetables and reported only a slight decrease in vitamin C content (less than 4%). These losses were explained by the authors by the oxidative effect of cold plasma and the potential vitamin C degradation through UV radiation generated by the plasma. Hou et al. [30] assessed the effect of cold plasma on the quality of blueberry juice. The authors used different treatment times (from 3 to 6 min) and suggested application of a shorter duration of cold plasma exposure in order to protect vitamin C (Table 1). Some authors noted an increase in vitamin C content in fruits and fruit juices caused by the action of cold plasma [31,32]. The oxidized form of ascorbic acid is dehydroascorbic acid, which is formed in the reaction with hydrogen, superoxide, hydrogen peroxide, or tocopheroxyl radical. Dehydroascorbate is regenerated by the dehydroascorbate reductase

enzyme, forming ascorbic acid. Cold plasma induces the hydrogenation of molecules, which can chemically transform dehydroascorbate into ascorbic acid, reversing the decay mechanism and increasing vitamin C content [33].

Color is an important quality characteristic of juices and a major factor affecting the sensory perception and consumer acceptance of foods. Various processing methods are used not only to increase the palatability of fruit or vegetable juices but also to prolong their shelf life. Cold plasma processing is an interesting alternative to the traditional food processing and preservation methods, due to its limited effects on changes in quality attributes in the juices. The effect of CAP on the color of the tomato juice is shown in Table 2.

Table 2. Effects of cold plasma processing on color attributes.

Time of Plasma Treatment (s)	Time Storage (Days)	Colour Attributes			ΔE
		L*	a*	b*	
0 (Control)		31.60 ± 0.09 ^{g}	6.68 ± 0.33 ^{h}	4.71 ± 0.22 ^{f}	NP
30	1	30.23 ± 0.02 ^{e}	4.94 ± 0.01 ^{d}	3.26 ± 0.01 ^{bcd}	2.65 ± 0.38 ^{b}
60		30.15 ± 0.03 ^{de}	4.87 ± 0.01 ^{d}	3.32 ± 0.02 ^{cd}	2.68 ± 0.37 ^{b}
120		30.07 ± 0.03 ^{d}	4.77 ± 0.04 ^{de}	3.26 ± 0.01 ^{bcd}	2.75 ± 0.38 ^{g}
300		29.77 ± 0.02 ^{a}	4.51 ± 0.02 ^{de}	3.09 ± 0.02 ^{be}	2.94 ± 0.37 ^{c}
600		29.78 ± 0.03 ^{a}	4.40 ± 0.02 ^{abc}	3.22 ± 0.03 ^{bc}	2.94 ± 0.37 ^{c}
0 (control)	10	29.75 ± 0.04 ^{a}	4.46 ± 0.01 ^{bc}	2.93 ± 0.02 ^{e}	NP
30		29.62 ± 0.03 ^{bc}	4.43 ± 0.02 ^{abc}	2.73 ± 0.02 ^{a}	0.72 ± 0.06 ^{d}
60		29.55 ± 0.04 ^{b}	4.15 ± 0.03 ^{a}	2.58 ± 0.01 ^{a}	1.14 ± 0.08 ^{f}
120		29.52 ± 0.02 ^{b}	4.18 ± 0.03 ^{ab}	2.70 ± 0.02 ^{a}	1.06 ± 0.09 ^{e}
300		29.36 ± 0.02 ^{f}	3.86 ± 0.06 ^{g}	3.86 ± 0.06 ^{a}	1.36 ± 0.12 ^{a}
600		29.70 ± 0.03 ^{ac}	2.96 ± 0.01 ^{f}	2.96 ± 0.01 ^{d}	1.38 ± 0.15 ^{a}

The results are expressed as a mean ± standard error. $^{a, b, c, d, e, f, g, h}$—statistically significant differences between the means ($p < 0.05$). NP = designation not performed.

Compared with the untreated juice, a significant decline in the brightness of the cold-plasma-treated samples was recorded on day 1 of the experiment. In turn, during storage (after 10 days), no statistically significant differences in the parameter L* values were observed in the samples exposed to cold plasma for 600 s, compared with the control product.

The chromaticity index a* changed with the changing plasma treatment time. The longer the time of the tomato juice CAP treatment, the lower the values of this parameter. The storage time exerted an effect on this attribute.

The chromaticity index b* changed after the cold plasma treatment on day 1. The CAP treatment was shown to reduce the yellowness parameter by approximately 32% in the juice treated for 600 s and the control product. On day 10, the chromaticity parameter b* was slightly lower than the values recorded on day 1 of the experiment. On the other hand, there was only a 1% difference in the values of this parameter in the control juice and the sample subjected to the longest cold plasma exposure (600 s).

The total color difference ΔE in the plasma-treated juices analyzed on day 1 was 2.65–2.94 (taking into account samples with extreme parameters), which indicates an average color deviation recognizable even by a non-expert. The total color difference was calculated for samples analyzed on day 10 as well. In the case of samples subjected to the 30 s cold plasma treatment, the ΔE values were below 1, indicating an indiscernible deviation, and between 1 and 2 indicating slight differences recognizable by a person experienced in discriminating color nuances.

Several studies reported various results of color changes after CAP treatment. For example, pomegranate juice became lighter after 7 min of plasma treatment with a total

color change between 2.0 and 3.0 [34]. Tests of color change in berry juice did not show a visual color difference between samples exposed to 2 and 4 min cold plasma treatment and samples processed thermally for 15 min, as the ΔE value ranged between 0.63 and 2.63 and reflected an unrecognizable or slightly noticeable color difference. However, the absolute color difference exceeded 3.5 in the 6 min plasma treatment and thermal treatment, indicating a clear difference in the color of the analyzed juices [30]. In the case of tomato juice, no significant differences in color parameters were found in different plasma exposure variants (30–120 s) [16].

3.2. Effect of CAP on Microorganisms Eradication

The presence of microorganisms in the unpasteurized tomato juice is responsible for its spoilage and, after several days of storage, makes it unsuitable for consumption. The lower the initial total number of microorganisms in the juice, the longer its shelf life. The initial total number of microorganisms in freshly prepared tomato juice depends on the quality of the vegetables used and the hygienic conditions of the juicing process. In the following days of refrigerated storage, the number of microorganisms increases due to their proliferation, which leads to juice spoilage and makes it endanger health in case of consumption. The total number of aerobic mesophilic microorganisms in edible juice should not exceed 3–4 $\log_{10}$ CFU/mL (Codex standards, 2005). It is assumed that the colony counts exceeding 4 $\log_{10}$ CFU/mL are responsible for the spoilage of the juice. In the present study, tomatoes were washed under warm running water before being used for juicing but were not sterilized. Likewise, the juicer was washed with washing-up liquid, without any germicides. The results showed that after just one day of juice storage under refrigeration conditions, the average number of mesophilic aerobic microorganisms in the tested samples was 3.22 $\log_{10}$ CFU/mL. With such a total number of microorganisms, the juice showed no signs of spoilage and was safe for consumption. After 3 days of refrigerated storage, a minor increase in the number of colonies formed by aerobic microorganisms (3.40 $\log_{10}$ CFU/mL) was observed, however, after 5 days of storage, their number increased to a high level exceeding 5 $\log_{10}$ CFU/mL, and after 10 days it was greater than 6 $\log_{10}$ CFU/mL. From the fifth day, a change in the organoleptic features of the juice was noted, and after 10 days, clear signs of deterioration were visible, including mold development on the surface of the juice. The content of yeast and mold after 1 day of storage was at a very low level (less than 0.5 $\log_{10}$ CFU/mL); however, their intensive proliferation during storage was noted, largely contributing to juice spoilage. After only 3 days their number increased to a level close to 3 $\log_{10}$ CFU/mL, and after 10 days it reached the value of 3.66 $\log_{10}$ CFU/mL. The results showed that, despite the low pH and low sugar content of tomato juice, the total number of aerobic microorganisms increased during refrigerated storage and after about 5 days made it unfit for consumption. The effectiveness of the treatment of freshly prepared juice samples with CAP in elimination of the total number of mesophilic aerobic microorganisms depended on the time of this treatment. The 30 s CAP treatment did not produce the desired effects as a reduction in the total number of microorganisms by less than 1 $\log_{10}$ CFU/mL was achieved, and after 10 days of storage there was a reduction of about 2 $\log_{10}$ CFU/mL in comparison to the untreated control (Figure 2). Similarly, the treatment with CAP for 60 and 120 sec had little effect of reducing the number of colonies counted after 1 and 3 days of storage, but the microorganisms in these samples proliferated much slower than in the control Thus, after 5 and 10 days of storage a reduction in the number of microorganisms by more than 2 $\log_{10}$ CFU/mL was observed compared to the control sample. The treatment with CAP for 300 s was found to be much more effective, the number of colony-forming units was reduced to an average of 1.83 $\log_{10}$ CFU/mL and during the following days of storage it was kept at a low level of just over 2 $\log_{10}$ CFU/mL, making the juice suitable for consumption even after 10 days of refrigerated storage. The greatest efficiency in erasing the microbial content was obtained by plasma treatment of the juice samples for 600 s. In this case, the number of colony-forming units dropped to less than 0.5 $\log_{10}$ CFU/mL and did not increase during the 10 days of storage (Figure 2). It can

therefore be concluded that CAP treatment for 600 s is an effective method of erasing the total number of aerobic microorganisms in tomato juice, extending its shelf life to 10 days, without heat treatment.

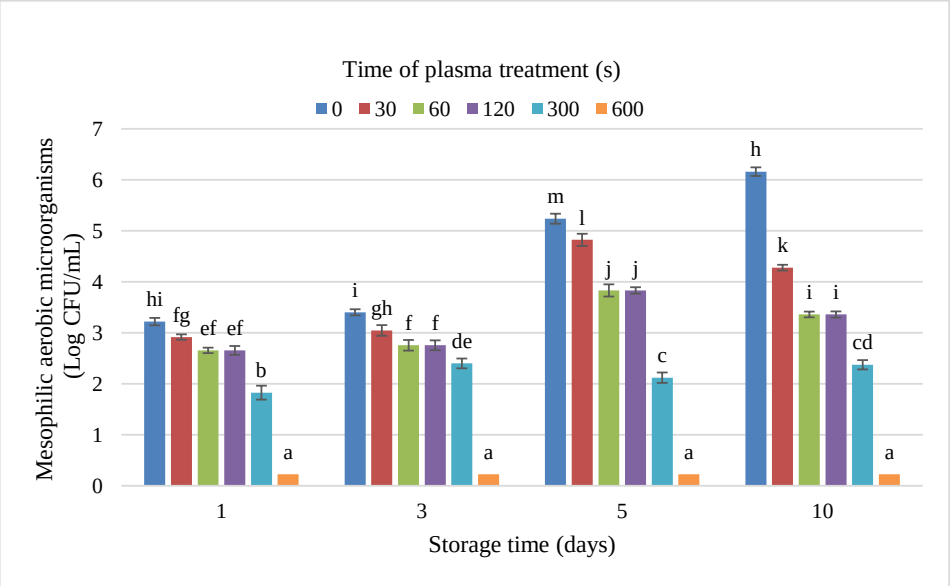

Figure 2. Number of mesophilic aerobic bacteria counts in control and CAP treated samples of unpasteurised tomato juice during refrigerated storage. Mean values (n = 4) with SD are shown a, b, c–statistically significant differences between the means ($p < 0.05$).

In case of erasing yeast and molds, the CAP treatment for 30, 60, and 120 s was much less effective. The number of yeasts and molds increased in these samples over the following days of storage, similar to the untreated control. In contrast, treatment with CAP for 300 and 600 s proved to be very efficient, since in these samples the number of yeasts and molds did not increase until the 10th day of storage and remained below 0.5 $\log_{10}$ CFU/mL Figure 3. Visually, no mold growth was observed on these samples until day 10 of storage.

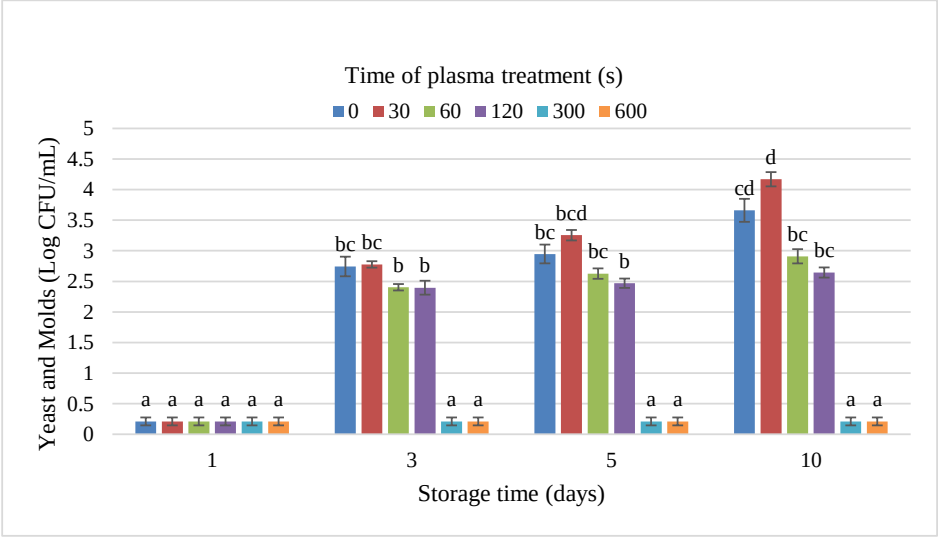

Figure 3. Number yeast and molds counts in control and CAP treated samples of unpasteurized tomato juice during refrigerated storage. Mean values (n = 4) with SD are shown. a, b, c—statistically significant differences between the means ($p < 0.05$).

As shown by the results of the latest studies published by other authors, the effectiveness of CAP in erasing sensitive strains of microorganisms in fruit juice is very high, even allowing a reduction by 8.2 $\log_{10}$ of the pathogenic strain of *Enterococcus faecalis*. Such results were obtained by CAP treatments for 300 s with plasma jet and 420 s with SDBD in pineapple juice [35]. The results of studies by the other authors also indicate the high efficiency of plasma in the reduction in pathogenic strains of microorganisms in fruit juices [8,11,30,36]. Dasan and Boyaci [16] investigated the inactivation effect of CAP on *E. coli*, and the highest significant reductions (after 120 s) were achieved in apple juice (4.02 ± 0.03 log CFU/ mL) followed by sour cherry (3.34 ± 0.09 log CFU/mL), while the values in orange (1.59 ± 0.17 log CFU/mL) and tomato juices (1.43 ± 0.22 log CFU/mL) were lower, which could be attributed to the food matrix. Our research results are in line with the mentioned report because 120 s CAP treatment was ineffective in erasing the total number of aerobic microorganisms as well as yeast and molds, but extending this time had satisfactory results in reducing microbial content.

3.3. Effect of CAP on the Structure of Tomato Juice

The microstructure of the juice was observed before and after plasma treatment. Figure 4 depicts the example of a parenchymal cell with organoids embed within the cell, which is surrounded by the juice homogenate. Treatment for 600 s did not cause distinctive fracturing of the cell wall and the cell membrane. The latter would result in the presence of lycopene crystals, chromoplasts, and cell organoids in the surrounding homogenate. Thus, plasma allows for relatively mild treatment of juice, which can help maintain its original textural properties.

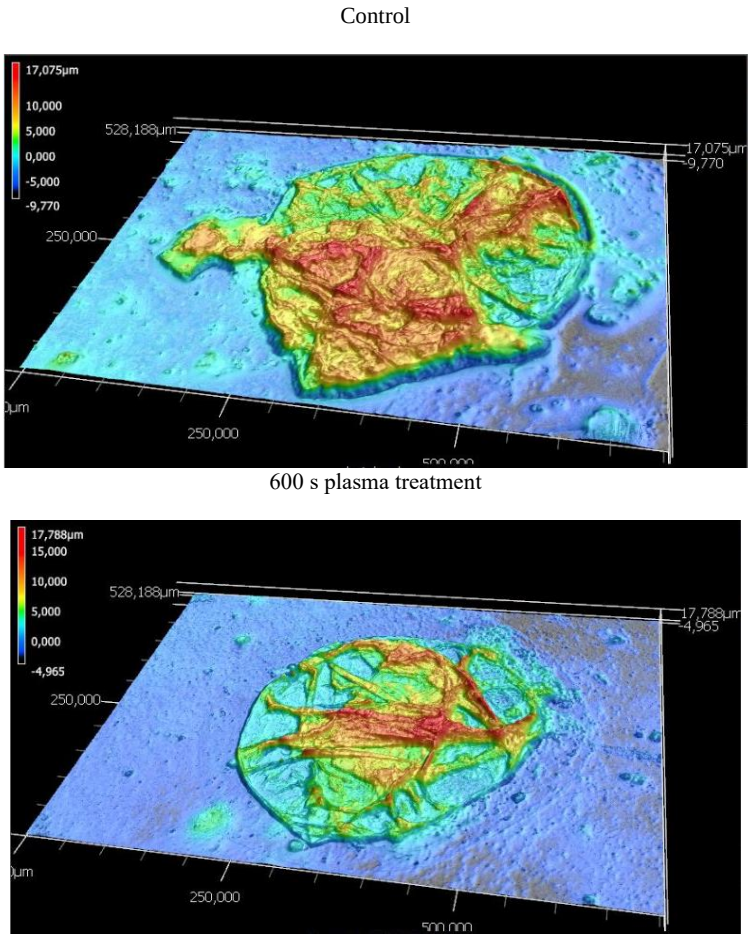

Figure 4. Comparison of the juice's sample before and after plasma treatment, depth analysis with Keyence VK-X1000 laser microscope.

4. Conclusions

Currently, the microbiological safety of food is the main task to be fulfilled by the food market. An extremely important issue is the ability to create systemic mechanisms ensuring an appropriate quality of freshly pressed fruit and vegetable juices, which are a particularly unstable food material. Products of this type should not only provide essential nutrients but should also exhibit high storage stability and availability facilitating trading in the retail chains.

Non-thermal air plasma generated in a Glide-arc reactor resulted in the significant improvement in the microbiological quality of tomato juice after just 300 s. Extending the treatment time to 600 s made it possible to further reduce the number of microorganisms to < 1 log CFU/mL, i.e., below the quantification limit. Properties, such as pH, Brix, lycopene, and vitamin C, were slightly increased by cold plasma treatment. During storage time, the CAP juice was more stable, while the untreated juice (control) showed gradual changes in physicochemical properties. The CAP treatment may better preserve the original color of the tomato juice, especially after time storage, since the samples tested on day 10 were characterized by the lowest absolute value of color difference (ΔE).

The application of the analyzed processing technique has many advantages in terms of both the microbiological quality and the nutritional value of products. The results presented in this study may contribute to the use of this method on an industrial scale. Despite its several aforementioned negative effects on the quality traits of products or the restrictions on its commercial use, the popularity of plasma treatment is growing rapidly, and the method arouses interest among technologists. Given the number of recent studies and the progress in plasma science, this technique has the potential to help food producers to improve product safety in the near future.

Author Contributions: Conceptualization, A.S.-W., J.P., M.K. (Michał Kwiatkowski) and P.T.; methodology, A.S.-W., A.S., J.P. and M.K. (Marta Krajewska); investigation, A.S.-W., J.P., M.K. (Michał Kwiatkowski), P.T., M.K. (Marta Krajewska), E.O.; data curation A.S.-W., J.P., A.S., E.G., E.O., M.K. (Marta Krajewska), N.M. and N.H.; writing—original draft preparation, A.S-W. and J.P.; writing—review and editing, A.S.-W., A.S., N.M., N.H.; visualization, J.P. and A.S.-W.; founding acquisition, J.P., A.S.-W. and E.G., supervision, J.P. All authors have read and agreed to the published version of the manuscript.

Funding: This research was founded from ZUL (Union of Lublin Universities) "INTERPROJEKT" grant no. INT/004/2022/I-N.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Data available upon justified request after contact with authors.

Acknowledgments: We would like to thank Wojciech Mika (Keyence International) for help in evaluation of morphology of the samples. We are grateful the fruitful discussions in the following cooperative initiatives: COST Actions CA19110 (Plasma applications for smart and sustainable agriculture), CA20114 (Therapeutical applications of Cold Plasmas); CEEPUS CIII-AT-0063, Inkubator Innowacyjności Politechnika Lubelska, and Polish-Slovak Bilateral Cooperation Programme (PlasmaBioAgro) PPN/BIL/2018/1/00065, SK-PL-18-0090.

Conflicts of Interest: The authors declare no conflict of interest.

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