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Scientific Research

Studying the Oxidant Activity and Optimization of Cold Plasma- Activated Water

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ABSTRACT

Activated water with cold-plasma has emerged as a non-toxic and environmentally friendly alternative, demonstrating its efficiency against a wide range of pathogens, including bacteria, viruses, and fungi. In this study, tap water was activated using cold plasma generator, employing a plasma reactor consisting of copper and steel electrons, with a voltage of 20 kV and a current of 3 mA at atmospheric air. To produce and optimization of activated water, experimental designs were conducted using Design Expert software version 12, following a Box-Behnken design. The factors were treatment time (0, 15, and 30 min), air injection velocity (0.5, 1, and 1.5 m/s), and storeage temperature (20, 4, and -20 °C). The characteristics of water, including hydrogen peroxide concentration, oxygen levels, total hardness, ozone, nitrate, nitrite, and chlorine, were measured at the days of 0, 1, 2, 3, and 6 to investigate the activation during storage period. The results indicated that all studied factors had a significant effect on the outcomes examined. The interaction effects of these factors also exhibited significant decreasing impact on the results in certain cases. Finally, it was determined that the storage temperature of the environment, prolonged treatment time with cold-plasma, and an air velocity of 1 m/s could yield activated water with superior oxidative properties. The optimized condition for producing activated water was identified as a treatment time of 23.5 min, 0.97 m/s air velocity, and storage temperature of 20°C.

1- Introduction

In recent years, the food industry has increasingly sought innovative solutions to enhance food safety, extend shelf life, and minimize the use of chemical additives. One promising technology that has received attention is plasma-activated water (PAW). This type of water is produced using cold plasma and contains reactive species such as radicals, ions, and ozone that can modify the physicochemical properties of water. These modifications have been shown to enhance the antimicrobial and antioxidant effects of water, making it suitable for various applications in food processing (Han et al., 2023; Herianto et al., 2021).

The increasing global demand for safer and more sustainable food processing methods has created a need for alternatives to conventional sanitation and preservation methods. Traditional methods often rely on chemical disinfectants, which may leave harmful residues and raise consumer concerns about food safety and environmental sustainability. In this context, PAW has emerged as a non-toxic and environmentally friendly alternative, and has demonstrated its efficacy against a wide range of pathogens, including bacteria, viruses, and fungi (Guo et al., 2017; Hadinoto et al., 2021; Julák et al., 2018; Rathore et al., 2021; Xiao et al., 2023). The use of PAW creates changes in the food industry that are in line with environmentally friendly processes and minimize the reliance on harmful chemicals while maintaining food safety standards (Guo et al., 2021; Xiang et al., 2022).

Recent studies have shown that PAW can effectively inactivate various pathogens responsible for foodborne illnesses. For example, Guo et al. (2017) found that PAW could reduce Saccharomyces cerevisiae CICC 1374 from the surface of grapes without significant changes in surface color and anthocyanin content after 30 min (Guo et al., 2017). Ali et al. (2021) showed that plasma-activated liquid (PAL) plus plasma-activated buffer solution (PABS) reduced the pesticides chlorothalonil and thiram in tomato fruit (Ali et al., 2021). Liu Chenghui et al. (2020) investigated the effect of PAW generated at 7 kHz with 6, 8, and 10 kV. They reported that PAW could reduce aerobic bacteria, molds, yeasts, and coliforms on the cut surface of Fuji apples (C. Liu et al., 2020) and colleagues (2020) found that the concentration of peroxynitrous acid (ONOOH) in PAW interacts with hydrogen peroxide to produce peroxynitric acid (O_2NOOH), which is then decomposed into O_2^- and $^1O_2$. These compounds are responsible for the enhanced antibacterial effects of PAW (Ma et al., 2020).

Research has also shown that PAW's unique composition can facilitate its application in various stages of food production, from washing fresh produce to disinfecting food processing equipment and surfaces. The versatility of PAW means that it can be integrated into existing food safety protocols, thereby enhancing overall hygiene without additional investment in new technologies (Al-Sharify et al., 2020; Milhan et al., 2022; Xiao et al., 2023). For example, in the processing of fresh-cut fruits and vegetables, PAW has shown promise in reducing microbial load while maintaining the quality and sensory properties of the products (Hou et al., 2021; Wong et al., 2023; Zhao et al., 2020; Zhou et al., 2018). Despite the promising properties of PAW, further research is needed to fully understand the optimal conditions for its production, such as the duration of cold water plasma treatment, plasma discharge conditions, and PAW storage temperature. In this study, the effect of some production and storage conditions on PAW activity will be investigated.

2-Materials and Methods

PAW Production:

A cold plasma generator device made at the Sari University of Agricultural Sciences and Natural Resources Growth Center (Figure 1) was used to produce PAW. This device consists of a cold plasma generator section and a sample storage section that is capable of producing cold plasma and direct contact of the sample with ionized air in this way. Using a plasma reactor consisting of copper and steel electrodes, a voltage of 20 kV and a current of 3 mA were generated with the help of atmospheric air and collided with the sample.

Figure 1- Cold- Plasma Emitter Machine

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The experimental design was implemented using Design Expert version 12 software in the form of a Box-Behnken design with the factors of plasma treatment time (0, 15, and 30 minutes), air injection speed (0.5, 1, and 1.5 m/s), and storage temperature of cold plasma-activated water (20, 4, and -20 °C) according to Table 1. In this study, urban water was used for activation. Because it has been determined in the review of the literature that the use of distilled water does not play a role in activation, and tap water has been used for this purpose so far (Han et al., 2023; Hoeben et al., 2019; Hou et al., 2021; Traylor et al., 2011).

Table 1- Box-Behnken Design for producing the PAW samples

Run	Cold- Plasma time (min)	Air Velocity (m/s)	Storage Temperature (°C)
1	30	0.5	0
2	0	1.5	0
3	15	0.5	-20
4	0	1	-20
5	30	1	-20
6	0	1	20
7	15	1	0
8	30	1	20
9	15	1.5	20
10	15	1	0
11	15	1	0
12	30	1.5	0
13	15	1	0
14	0	0.5	0
15	15	1.5	-20
16	15	1	0
17	15	0.5	20

Determination of water chemical properties

Before use, water properties including hydrogen peroxide (Karizab 4436 kit), oxygen (Arak Chemical Eng. Co. AK 3060 kit), total hardness (Arak Chemical CO.), ozone (Arak Chemical Eng. Co. 3096), nitrate (Arak Chemical Co. 3020), nitrite (Arak Chemical Co. 3015) and chlorine (Arak Chemical CO.) were measured. These properties were also tested during days 1, 2, 3 and 6.

Statistical Analysis

All tests were performed in triplicate and statistical analysis was performed using SPSS v.20 software using analysis of variance with Tukey test to determine significance. A *p* value of less than 0.05 was considered statistically significant.

3.Results and Discussion

Table 2 shows the statistical analysis of the effects of the studied factors of cold plasma treatment time, inlet air velocity, holding time and holding temperature of cold plasma activated water at the 5% level ($p < 0.05$). According to this table, the effect of cold plasma time (0, 15 and 30 minutes) on nitrate and total hardness responses was significant. The inlet air velocity (0.5, 1 and 1.5 m/s) was significant on nitrite and total hardness. The holding temperature (20, 4 and -20 °C) was significant on total hardness and finally the holding days (1, 2, 3 and 6 days) had a significant effect on all factors except nitrite and ozone. This finding shows that the studied factors were able to have a significant effect on the quality and activity of active species produced during cold plasma treatment.

Table 2- ANOVA analysis for the effect of factors on studied results

Cold-Plasma Time (min)						
		Sum of Squares	df	Mean Square	F	Sig.
Nitrate	Between Groups	204.118	2	102.059	3.626	0.032

	Within Groups	1829.764	65	28.150		
	Total	2033.882	67			
	Between Groups	0.038	2	0.019	2.067	0.135
Nitrite	Within Groups	0.593	65	0.009		
	Total	0.631	67			
	Between Groups	0.054	2	0.027	2.267	0.112
Ozone	Within Groups	0.775	65	0.012		
	Total	0.829	67			
	Between Groups	0.076	2	0.038	2.081	0.133
Chloride	Within Groups	1.187	65	0.018		
	Total	1.263	67			
	Between Groups	289808.563	2	144904.281	7.258	0.001
Hardness	Within Groups	1297664.188	65	19964.064		
	Total	1587472.750	67			
	Between Groups	23.721	2	11.860	0.329	0.721
Oxygen	Within Groups	2345.135	65	36.079		
	Total	2368.856	67			
	Between Groups	111.890	2	55.945	1.175	0.315
H2O2	Within Groups	3094.625	65	47.610		
	Total	3206.515	67			
Air Velocity (m/s)						
		Sum of Squares	df	Mean Square	F	Sig.
	Between Groups	11.118	2	5.559	0.179	0.837
Nitrate	Within Groups	2022.764	65	31.119		
	Total	2033.882	67			
	Between Groups	0.045	2	0.023	2.505	0.089
Nitrite	Within Groups	0.586	65	0.009		
	Total	0.631	67			
	Between Groups	0.026	2	0.013	1.064	0.351
Ozone	Within Groups	0.803	65	0.012		
	Total	0.829	67			
	Between Groups	0.060	2	0.030	1.632	0.204
Chloride	Within Groups	1.202	65	0.018		
	Total	1.263	67			
	Between Groups	154371.924	2	77185.962	3.501	0.036
Hardness	Within Groups	1433100.826	65	22047.705		
	Total	1587472.750	67			
	Between Groups	25.841	2	12.920	0.358	0.700
Oxygen	Within Groups	2343.015	65	36.046		
	Total	2368.856	67			
	Between Groups	5.765	2	2.882	0.059	0.943
H2O2	Within Groups	3200.750	65	49.242		
	Total	3206.515	67			
Storage Temperature (℃)						
		Sum of Squares	df	Mean Square	F	Sig.
	Between Groups	10.132	2	5.066	0.163	0.850
Nitrate	Within Groups	2023.750	65	31.135		
	Total	2033.882	67			
	Between Groups	0.011	2	0.006	0.577	0.565
Nitrite	Within Groups	0.620	65	0.010		
	Total	0.631	67			
	Between Groups	0.039	2	0.020	1.615	0.207
Ozone	Within Groups	0.790	65	0.012		
	Total	0.829	67			
	Between Groups	0.035	2	0.018	0.936	0.397
Chloride	Within Groups	1.227	65	0.019		
	Total	1.263	67			

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Hardness	Between Groups	382001.569	2	191000.785	10.299	0.000
	Within Groups	1205471.181	65	18545.710		
	Total	1587472.750	67			
Oxygen	Between Groups	94.519	2	47.259	1.351	0.266
	Within Groups	2274.337	65	34.990		
	Total	2368.856	67			
H2O2	Between Groups	0.265	2	0.132	0.003	0.997
	Within Groups	3206.250	65	49.327		
	Total	3206.515	67			
		Storage Days				
		Sum of Squares	df	Mean Square	F	Sig.
Nitrate	Between Groups	608.588	3	202.863	9.109	0.000
	Within Groups	1425.294	64	22.270		
	Total	2033.882	67			
Nitrite	Between Groups	0.035	3	0.012	1.258	0.296
	Within Groups	0.596	64	0.009		
	Total	0.631	67			
Ozone	Between Groups	0.051	3	0.017	1.404	0.250
	Within Groups	0.778	64	0.012		
	Total	0.829	67			
Chloride	Between Groups	0.656	3	0.219	23.044	0.000
	Within Groups	0.607	64	0.009		
	Total	1.263	67			
Hardness	Between Groups	285850.750	3	95283.583	4.685	0.005
	Within Groups	1301622.000	64	20337.844		
	Total	1587472.750	67			
Oxygen	Between Groups	664.995	3	221.665	8.326	0.000
	Within Groups	1703.860	64	26.623		
	Total	2368.856	67			
H2O2	Between Groups	2078.868	3	692.956	39.329	0.000
	Within Groups	1127.647	64	17.619		
	Total	3206.515	67			

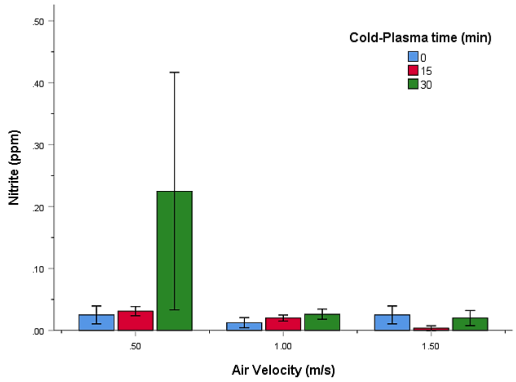
Cold plasma generating systems at atmospheric pressure and with normal air have often been used to produce activated water, because these systems are easy to design and inexpensive to maintain. However, it should be noted that the characteristics of the plasma activated water production process, including power, voltage, and gas type, vary among different treatments. Many studies have focused on investigating the effects of PAW production factors (Guo et al., 2021; Han et al., 2023; Zhou et al., 2020). For example, Qi et al. (2018) investigated the effects of voltage and treatment time on PAW production by distilled water and reported that with increasing voltage in PAW production, its microbial population reduction property also increases (Qi et al., 2018). Zhao et al. (2020) also reported that increasing the voltage and exposure time of water will increase the microbial inactivation property of PAW (Zhou et al., 2020). In this study, it was found that the studied factors had an effect on the oxidative properties of plasma-activated water. Also, the interaction effect of plasma treatment time and the factors of storage day, storage temperature, and inlet air velocity is shown in Figures 1 to 3.

Effect of cold plasma treatment time and inlet air speed on the amount of active compounds

One of the factors affecting the effectiveness of PAW is the gas flow rate (Wong et al., 2023; Xiao et al., 2023). According to studies, the gas flow rate has not always shown a positive relationship with the oxidative activity of PAW. In this study, Figure 1 shows that the interaction effect of inlet air speed and plasma treatment time did not cause significant changes in the amount of active oxidative compounds in PAW. Although the inlet air speed factor and plasma treatment time factor separately have been able to show a strong significant effect on the production of these compounds, their overall interaction has been significant in some conditions. For example, increasing the plasma treatment time and increasing the inlet air speed has been able to have a significant difference in hardness reduction, which confirms the results of research in the field of wastewater and water treatment by cold plasma. Air velocity had a significant reducing effect on hardness and ozone at 15 and 30 minutes of cold plasma treatment.

When PAW is generated, various processes occur at the water-plasma interface, such as the transfer of active species generated in the plasma to water and the occurrence of chemical interactions between active species and water molecules (Wende et al., 2019). The discharges generated in water are very active and transient, and the active species annihilation processes occur, which often occur in strong electric fields, leading to the formation of short-lived species such as hydroxyl ions and dissolved hydrated electrons (Khlyustova et al., 2019). Subsequent rapid reactions between hydroxyl ions and dissolved hydrated electrons lead to the production of active species such as ozone and hydrogen peroxide. Liu et al. (2020) reported that the active oxygen species decreased with increasing inlet air velocity. They attributed this to the fact that the air carried a large portion of the gas phase of the active species from the chamber, reducing the ability of those active species to dissolve in water (K. Liu et al., 2020).

The decrease in water hardness due to increased cold plasma treatment time can be attributed to the production of free radicals in water that can decompose calcium and magnesium ions or produce gases that interact with these ions, making them unavailable (Kamgang-Youbi et al., 2009; Zhao et al., 2020).



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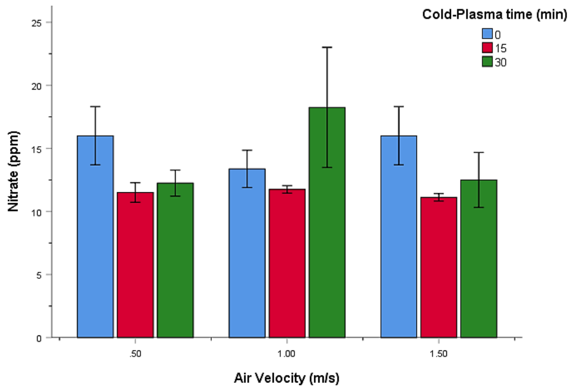
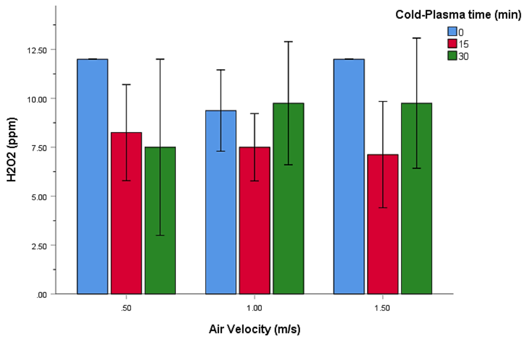
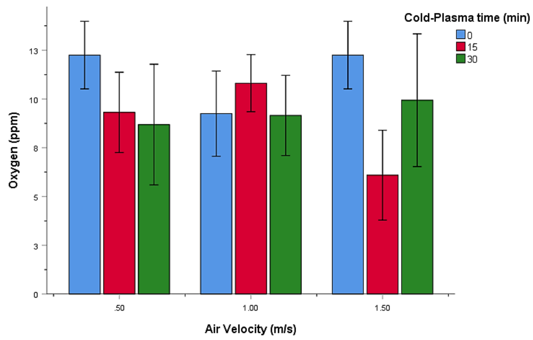


(b)



(c)



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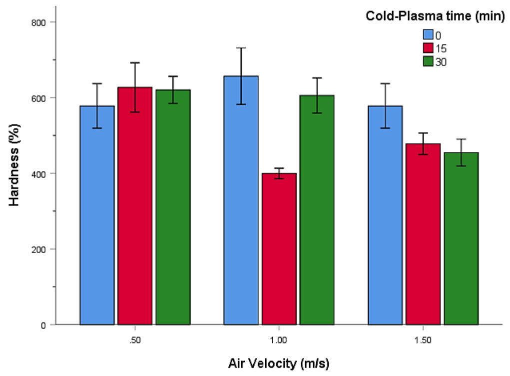

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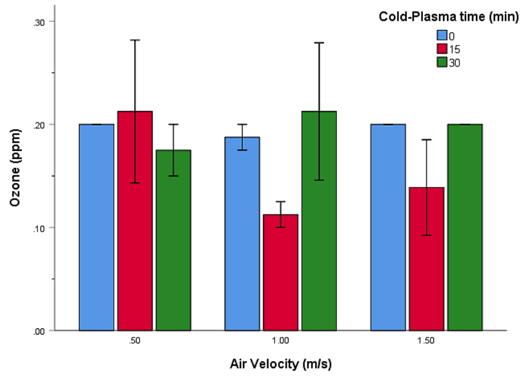
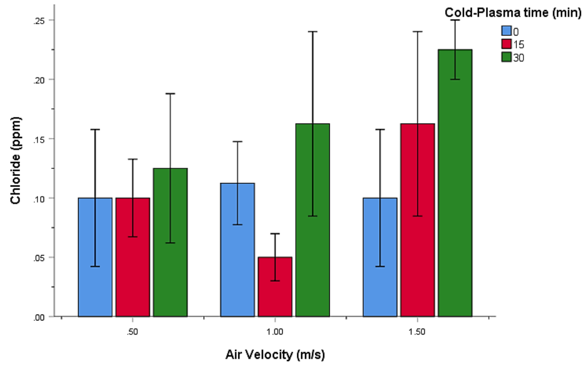


(f)



(g)

Figure 1- The interaction effect of cold-plasma time and inlet air velocity on Nitrite (a), Nitrate (b), H_2O_2 (c), Oxygen (d), Hardness (e), Ozone (f), and Chloride (g) contents

Effect of storage time on the amount of active compounds

According to Figure 2, by changing the cold plasma treatment time, the amount of oxidant compounds in the samples has changed significantly. These changes are clearly visible during the storage days and the changes of each sample in each day are quite significant. Among these compounds, hydrogen peroxide, nitrate, nitrite and ozone are considered indicators of the antimicrobial activity of PAW (Zhang et al., 2013). By monitoring the changes of these compounds with respect to the cold plasma treatment time, it was found that on the first day, with increasing CP time, the production of hydrogen peroxide, nitrate and ozone increased, but on the second day of storage, their levels decreased and in the case of nitrate and ozone, they remained constant until the last day of storage. The rate of hydrogen peroxide reduction in the samples that received 30 minutes of cold plasma treatment was very high during six days of storage. In the case of chlorine, increasing the plasma treatment time on the first day increased its level, but in the following days of storage, the chlorine level decreased sharply, independent of the cold plasma treatment time. Niquet et al. (2018) reported that with increasing the cold plasma treatment time, the hydrogen peroxide concentration also increased, and this stability was maintained to some extent during storage (Niquet et al., 2018).

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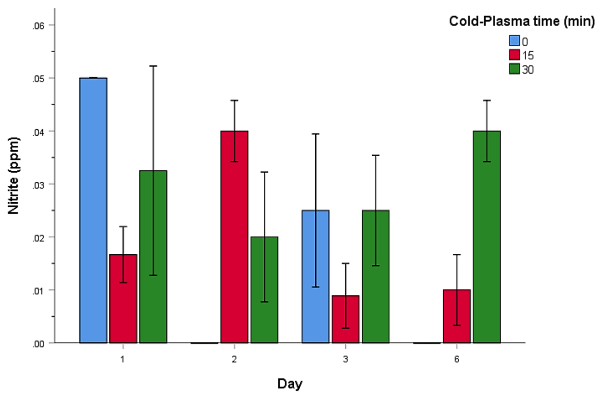
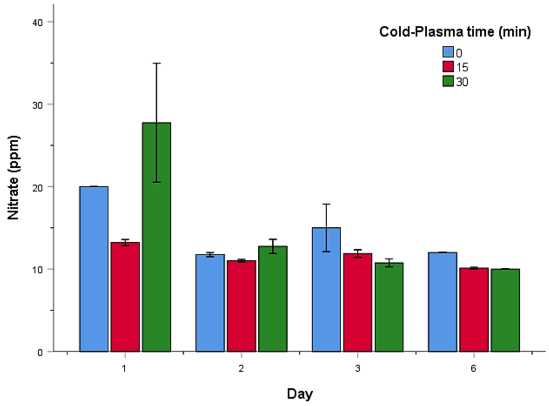
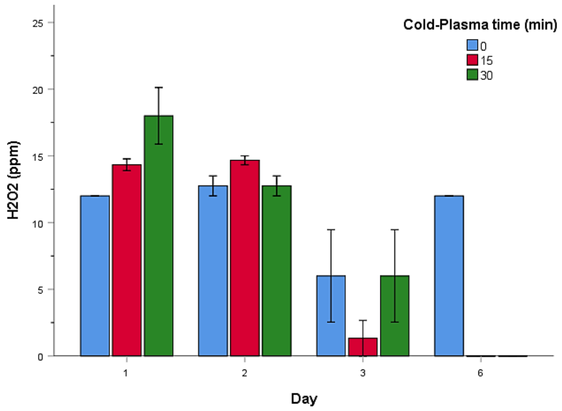

Cold-Plasma time (min)
0
15
30
Nitrite (ppm)
.00
.01
.02
.03
.04
.05
.06
1
2
3
6
Day

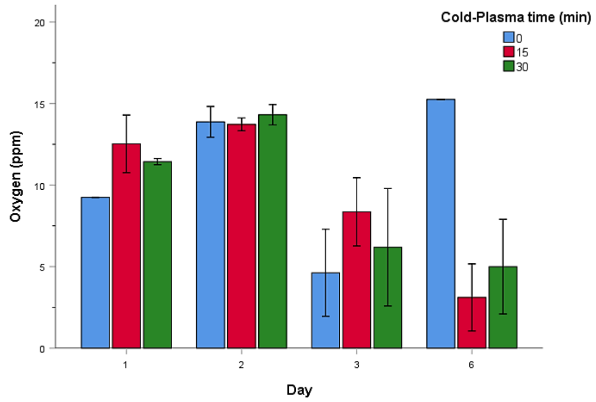
(a)


Cold-Plasma time (min)
0
15
30
Nitrate (ppm)
0
10
20
30
40
1
2
3
6
Day

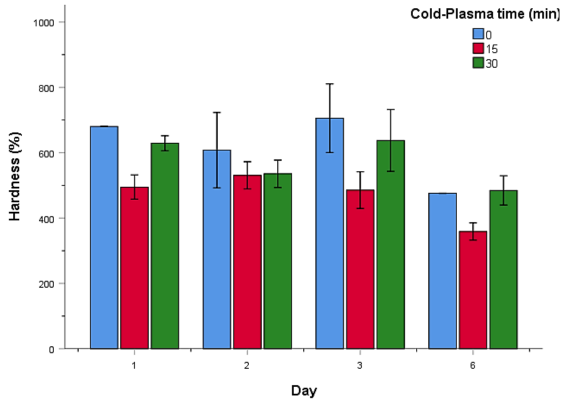
(b)


Cold-Plasma time (min)
0
15
30
H2O2 (ppm)
0
5
10
15
20
25
1
2
3
6
Day

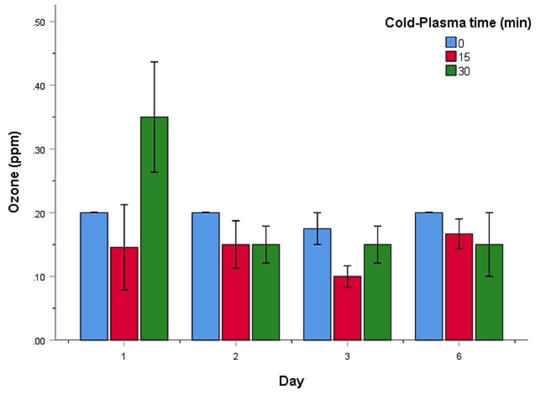
(c)


Cold-Plasma time (min)
0
15
30
Oxygen (ppm)
0
5
10
15
20
1
2
3
6
Day

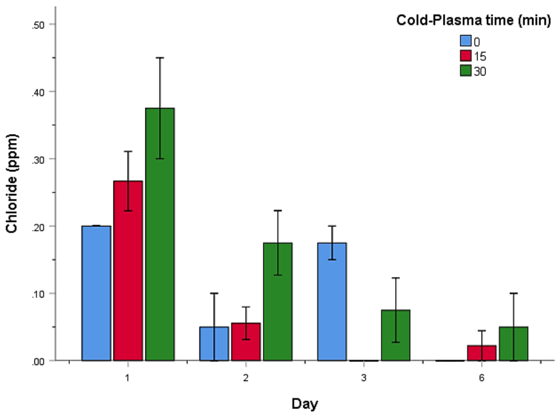
(d)


Cold-Plasma time (min)
0
15
30
Hardness (%)
0
200
400
600
800
1000
1
2
3
6
Day

(e)


Cold-Plasma time (min)
0
15
30
Ozone (ppm)
.00
.10
.20
.30
.40
.50
1
2
3
6
Day

(f)

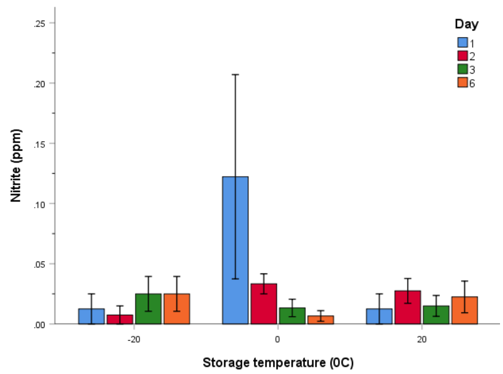

Cold-Plasma time (min)
0
15
30
Chloride (ppm)
.00
.10
.20
.30
.40
.50
1
2
3
6
Day

(g)

Figure 2- The interaction effect cold-plasma time and storage duration of PAW on Nitrite (a), Nitrate (b), H_2O_2 (c), Oxygen (d), Hardness (e), Ozone (f), and Chloride (g) contents

Effect of storage time and temperature on the amount of active compounds

Another important factor in the oxidative quality of PAW is its storage temperature. One of the important ideas that can be of great importance in the food industry today is the possibility of producing and storing this water for a longer period of time. In particular, whether this water can be stored and transported frozen or not. For this reason, in this study, the oxidative status of PAW was investigated at three temperatures: ambient, refrigerated, and frozen. Figure 3 shows the interaction between storage time and storage temperature of PAW samples. According to this figure, the amount of nitrite showed a significant decrease during refrigerated storage. However, nitrate decreased significantly at all storage temperatures with increasing storage time. Also, the amount of hydrogen peroxide was the highest on the first and second day at all storage temperatures, but it showed a decreasing trend at all temperatures until the sixth day. These results are consistent with the findings of (Zheng, 2017). Oxygen content did not show a significant relationship with the interaction of storage time and temperature, but total hardness increased at freezing temperature and decreased significantly at refrigerator and ambient temperatures until the last day. Ozone content, which is one of the most important indicators of PAW activity, increased significantly in samples stored at refrigerator temperature until the sixth day, but its changes decreased at the other two temperatures. Chlorine also showed a decreasing trend at all storage temperatures. Ozone dissolved in water is not very stable, but its presence depends on the hydroxyl radical (Leuratti et al., 1998). Hydroxyl radicals are short-lived reactive species that have high oxidation and reduction potential and react with organic substances in the presence of water to create new radicals that cause further reactions (Ayala et al., 2014; Herianto et al., 2021; Laurita et al., 2015).



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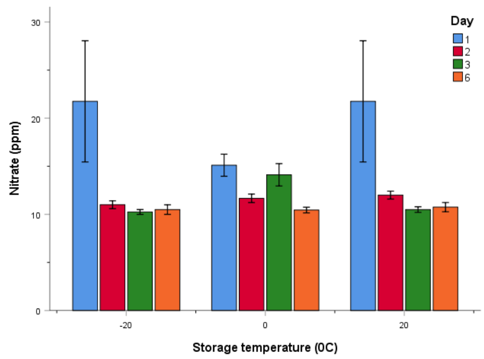

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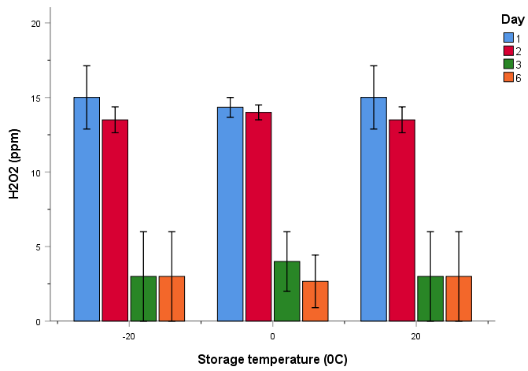

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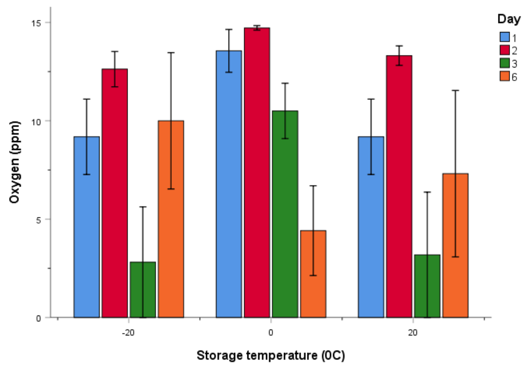

(d)

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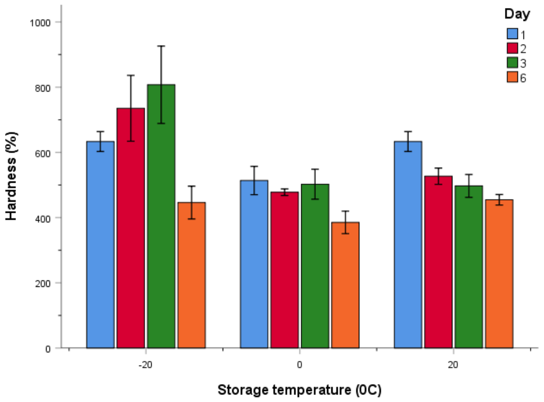
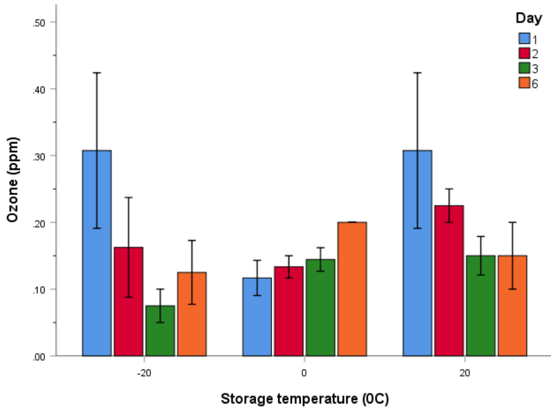
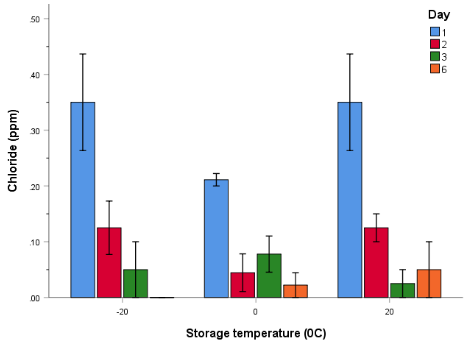


(e)



(f)



(g)

Figure 3- The interaction effect of storage time and temperature of PAW on Nitrite (a), Nitrate (b), H_2O_2 (c), Oxygen (d), Hardness (e), Ozone (f), and Chloride (g) contents

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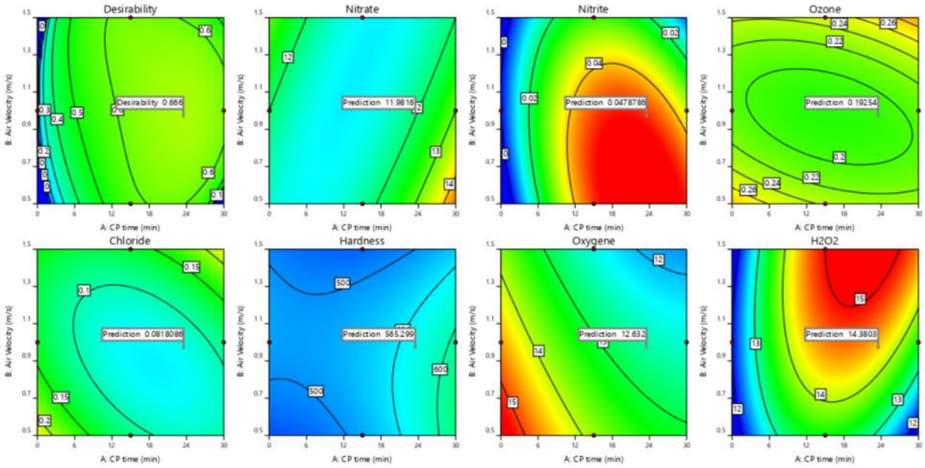
Optimization of PAW Production

Due to the effect of interacting factors on the oxidative activity of PAW, optimizing the processing conditions with respect to the application of PAW is of great importance; therefore, this work was carried out with the help of Design Expert version 12 software with the aim of maximizing the oxidative activity of PAW and minimizing the hardness, and the results are shown in Figure 4. Accordingly, the best processing conditions to achieve the aforementioned goal with a sensitivity level of 3 were a treatment time of 23.5 minutes with cold plasma, an air velocity of 0.97 m/s, and a storage temperature of 20 °C. In this way, it is possible to produce activated water with cold plasma with the highest efficiency.



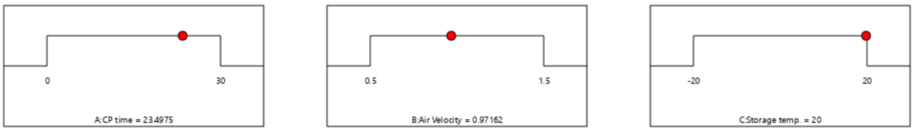


Figure 4- The optimization of PAW production

4.Conclusion

This study showed that PAW as a non-toxic and environmentally friendly alternative can be investigated and produced. Using a cold plasma generator and designing experiments and optimizing conditions will lead to the production of water with the highest activity and the lowest hardness. Also, the time of treatment with cold plasma, the speed of air entering the system, and the storage temperature of PAW will have significant effects on its properties, which can be planned for the preparation and storage of PAW according to the type of application expected from it. These results can form the basis for future applications of cold plasma activated water to maintain hygiene in the food and agricultural industries.

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مقاله علمی_پژوهشی

بررسی فعالیت اکسیدانی و بهینه‌سازی تولید آب فعال شده با پلاسمای سرد

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چکیده

آب فعال شده با پلاسمای سرد به‌عنوان یک جایگزین غیرسمی و دوستدار محیط‌زیست معرفی و کارایی خود در برابر طیف گسترده‌ای از عوامل بیماری‌زا از جمله باکتری‌ها، ویروس‌ها و قارچ‌ها را نشان داده است. در این پژوهش از آب شهری برای فعال‌سازی توسط دستگاه مولد پلاسمای سرد با استفاده از یک رآکتور پلاسما شامل الکترودهای مسی و فولادی، ولتاژ ۲۰ kV جریان ۳ mA با کمک هوای اتمسفر استفاده شد. برای تولید آب فعال شده، طرح آزمایشات توسط نرم‌افزار دیزاین اکسپرت ورژن ۱۲ در قالب طرح باکس–بنکن با فاکتورهای زمان تیمار با پلاسما (۰، ۱۵ و ۳۰ دقیقه)، سرعت تزریق هوا (۰/۵ m/s، ۱ و ۱/۵) و دمای نگهداری آب فعال شده با پلاسمای سرد (°C ۲۰، ٤ و ۲۰–) اجرا شد. قبل از استفاده، ویژگی‌های آب از جمله میزان پراکسید هیدروژن، اکسیژن، سختی کل، ازن، نیترات، نیتریت و کلر اندازه‌گیری شد. این ویژگی‌ها طی مدت روزهای ۱، ۲، ۳ و ٦ نیز مورد آزمون قرار گرفتند. نتایج نشان دادند تمام فاکتورهای موردمطالعه بر نتایج مورد بررسی اثر معنادار داشتند. اثر متقابل این فاکتورهای نیز در برخی موارد اثر معنادار کاهشی و یا افزایش بر نتایج داشتند. به‌طورکلی مشخص شد دمای نگهداری محیط، زمان تیمار بیشتر با پلاسمای سرد، و سرعت هوای ۱ m/s می‌تواند آب فعال با ویژگی‌های اکسیدانی بهتری را تهیه کند. شرایط بهینه تولید آب فعال شده نیز عبارت بود از زمان تیمار ۲۳/۵ دقیقه با پلاسمای سرد، سرعت هوای ۰/۹۷ m/s و دمای نگهداری ۲۰°C.