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Research Article

Evaluation of Cold Plasma Effect on Shelf Life, Physicochemical and Organoleptic Properties of Cucumber (*Cucumis* Sativus Var. Negin)

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Abstract

Non-thermal plasma is a new technique to reduce the microbial load in fresh fruits and vegetables, which can decontaminate the surfaces of fresh food products. This research used the atmospheric pressure jet discharge plasma of argon gas to treat cucumber samples. The treatments included two power levels (voltage of 17 volts with a current intensity of 2.66 amps and voltage of 10 volts with a current intensity of 0.86 amps) and four-time levels of plasma application (0, 2, 4, and 6 minutes) on the surface of cucumber samples. Cucumbers' microbial, qualitative, and sensory characteristics were measured after plasma application and during storage at 250C in three periods, the first, fifth, and tenth days after plasma application. The results of the microbial analysis in plasma-treated samples with the machine's high power showed a decrease of 2.59 logarithmic cycles in the total count and 1.388 logarithmic cycles in mold and yeast. No significant difference was observed between the tissue hardness and color indices of the treated samples immediately after plasma application and the corresponding values of the control sample in both powers. Examining the quality indicators of cucumber samples during storage showed the superiority of the treated samples compared to the control samples. The sensory evaluation of cucumber samples indicated that plasma had no adverse effect on the sensory characteristics of cucumbers. The aroma characteristics and general acceptability of the plasma-treated samples were significantly better than the control samples during the storage period. Based on the results, cold plasma is a new method for food processing. Due to its non-thermal nature, this technique can be a suitable alternative to other techniques used for the decontamination, pasteurization, and sterilization of food products, especially heat-sensitive products.

Keywords: Sensory evaluation; Microbial load; Cold plasma, Cucumber, Shelf life

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Introduction

The statistics provided by the Food and Agriculture Organization of the United Nations (FAO), in 2022, about 40 to 50 percent of the global fruits and vegetables will be lost during transportation and storage after harvest. They go away mainly due to microbial spoilage, moisture loss, rotting, and discoloration [17]. Microbial contamination is one of the critical issues in the food industry. The risks associated with the transmission of foodborne pathogenic microorganisms have become a global concern in the food industry. In 2020, European Union member states reported 3,086 foodborne disease outbreaks. There are many reports that raw vegetables and fruits cause the transmission of pathogens. Pathogenic agents often associated with these epidemics include *Salmonella*, *Escherichia coli*, and *Listeria monocytogenes* [7]. The outbreak of diseases related to health conditions associated with consuming food products requires research to achieve more efficient decontamination techniques [5,10]. Many treatments have been developed to increase the shelf life of vegetables after harvest. Controlling temperature, maintaining a controlled atmosphere, and using waxes and other coatings, including natural antimicrobial compounds, are essential to maintain the quality of vegetables after harvest [6]. Many treatments have been developed to increase the shelf life of vegetables after harvest. Controlling temperature, maintaining a controlled atmosphere, and using waxes and other coatings, including natural antimicrobial compounds, are essential to maintain the quality of vegetables after harvest [3]. Antibiotics are also effective agents for controlling bacterial infection to a certain extent, but they have the disadvantages of producing antibiotic-resistant bacteria and destroying microbial ecosystems [25].

Non-thermal disinfection methods such as gamma rays and fumigation with ethylene oxide can also help deactivate the microbial flora of fruits and vegetables. But ethylene oxide is prohibited in the European Union due to its carcinogenic effect. The limitation of conventional techniques has prepared the ground for developing other strategies [1,24]. Among the various methods used to reduce the microbial load or inactivate microorganisms, cold plasma is preferred due to its numerous potential advantages, such as its non-toxic nature, low operating costs, significant reduction of water consumption during disinfection processes, and the possibility of Its use for various food products have attracted a lot of attention [31]. Plasma is produced by applying an electric field to a neutral gas. Plasma is a state of ionized gas containing ions, electrons, ultraviolet rays, and reactive species such as radicals, atoms, and excited molecules capable of inactivating microorganisms [25]. Recently, cold plasma at atmospheric pressure has been considered a food disinfectant because cold plasma is a dry, non-thermal technology without the need for chemicals, which can work continuously at atmospheric pressure [4].

The cucumber, with its scientific name (*Cucumis sativus* L.), is from the family of gourds and is the most famous vegetable that is used fresh and processed. This plant hosts many pathogens caused by soil and air. Fungi, bacteria, viruses, nematodes, Rickettsia, and parasitic flowering plants are the pathogenic agents that affect the plant at different stages of growth and development. Diseases such as cucurbit angular leaf spot, false powdery mildew, plant wilt, surface powdery mildew, contamination caused by nematodes, and cucumber mosaic virus are among the most important diseases of cucumber [28,33]. Therefore, this research aimed to investigate the effect of cold plasma on the physicochemical, sensory, and microbiological characteristics of cucumbers during ten days of storage at room temperature.

Materials and Methods

The tested cucumbers were purchased from a Mashhad, Khorasan Razavi province greenhouse, Iran. Several 12 cucumbers were randomly selected and tested to determine the essential physicochemical characteristics (texture, color, taste, smell, and marketability) and microbial profile (total count and mold and yeast count).

Twenty-four cucumber samples without applying cold plasma were placed as a control sample in the same package in an incubator with a temperature of 25⁰C. Sixty cucumber samples were also packed and transferred to an incubator with a temperature of 25⁰C after applying experimental treatments to check the storage life. The pieces are treated by atmospheric pressure jet discharge plasma (manufactured by Nik Plasma Company of Iran) in the device's highest and lowest power modes. The voltage of 17 volts and current intensity 2.66 amps and voltage 10 V and current intensity 0.86 amps with argon gas below Sterile hoods were treated at the desired times. The treated samples were immediately packed in containers and transferred to an incubator at 25°C. The samples were examined in terms of physicochemical, sensory, and microbial characteristics in 5-day intervals.

Measurement of Microbial Characteristics

Plate count agar (PCA) and Potato Dextrose Agar (PDA) sterile culture media were used to evaluate the microbial count of the samples, including total count and mold and yeast count (ISO 21527-1:2008; ISO 4833-1:2013; Jidda & Muhammad, 2017).

Measurement of Physicochemical Properties

Texture hardness: The hardness of the cucumber texture was measured by a texture analyzer (model STM-20).Four parts of the cucumber were pressed using a cone head that penetrates to a depth of 10 mm at a speed of 60 mm/min and a pressure of 50 N/mm. The number obtained from the software showed the hardness of the texture [23].

Color: The color parameters (L*, a*, and b*) of the samples were measured with a Hunter Lab colorimeter (American model) [30].

Sensory evaluation: Twelve trained people evaluated the product›s sensory properties in color, taste, texture, hardness, and overall acceptance of the samples.

Statistical Method

Experiments were conducted in a completely randomized factorial design in 3 replications. Statistical analysis of data was analyzed using SPSS software. Duncan's test was used to compare the mean and examine the effects of the treatments ($p \leq 0.05$).

Results and Discussion

Microbial Load Assessment

As shown in (Figure 1), the effect of the plasma production device's power and the plasma application's duration on the number of mold and yeast and the total count was significant at the 5% level.

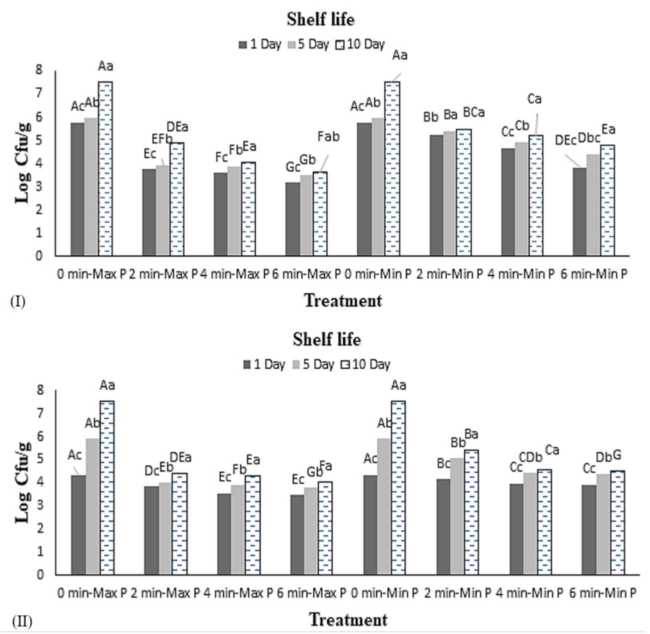

Figure 1: Comparison of the averages of the effect of the power of the plasma device and the duration of plasma application on **(I)** total count and **(II)** mold and yeast count during the storage period. (Lowercase letters refer to comparing one treatment on different days, and upper-case letters refer to comparing treatments in one day). These means with at least one similar letter do not have a significant difference at the probability level of 0.05 with Duncan's test.

The comparison of the microbial count averages of the samples showed that the total number of microorganisms, mold, and yeast decreased with the increase in the power of the device and the duration of plasma application. As shown in (Figure 1), the most significant reduction of microorganisms was in the extended period of plasma application and higher power. The results obtained in this field are consistent with the results of research by Bermúdez-Aguirre *et al.* and Lacombe *et al.* These researchers also used different periods to treat blueberry aerobic bacteria with plasma. In all treatments, the total number of bacteria decreased significantly ($P<0.05$) by increasing the duration of plasma application. So that immediately after the treatment, the reduction of the microbial load was about 1.7 logarithmic cycles [3,16]. [35] also reported that high-voltage electric discharge plasma treatment (voltage 30 kV, frequency 40 Hz, and 25 °C) for 2 minutes reduced *Escherichia coli* by 4.8 logarithmic units in tangerine juice. Bermúdez-Aguirre et al. reported the ability of atmospheric pressure plasma to inactivate microorganisms on the surface of lettuce, carrot, and tomato. The plasma generator system used in this research had a voltage of 3.95 - 12.83 kV and a frequency of 6 kHz. For the inactivation of lettuce, carrot, and tomato microorganisms, the plasma application duration was between 30 seconds and 5 minutes. At the end of the experiment, the researchers reported a direct correlation between the reduction of microorganisms and the time of plasma application [3].

Our research showed that the microbial load and the number of mold and yeast for cucumber samples before plasma application were 5.762 Log cfu/g and 4.858 Log cfu/g, respectively. Using argon gas plasma reduced the total microbial load by 2.59 logarithmic cycles, and the microbial load of mold and yeast was decreased by 1.388 logarithmic cycles. (Table 1) showed a more significant reduction in mold and yeast than bacteria.

Table 1: The results of counting the microbial load on the surface of cucumber samples before and after applying atmospheric pressure plasma with argon gas.

Microorganism	Initialmicrobialload (Log cfu/g)	Microbial load after 6 minutes of plasma application (Log cfu/g)
Total microorganisms	5.762	3.172
Mold and yeast	4.858	3.47

Park etal. used an argon microwave plasma system to inactivate bacteria and fungi. The results showed that all bacteria used in this study were sterilized entirely within 20 seconds and all fungi within 1 second of plasma application. Based on these results, it was observed that there is a significant difference between the sensitivity of bacteria and fungi to plasma [26]. This difference seems to be caused by the nature of the cell wall of fungi and bacteria. Fungi have a hyphae-like structure, so more are exposed to plasma than bacteria. Reactive species and UV waves produced by plasma can diffuse through the membrane in the form of solid chemical and physical reactions faster in fungi than in bacteria and directly react with biological substances inside the cell. Therefore, fungi require a significantly shorter time of plasma application for sterilization than bacteria. Mao et al. have claimed that membrane disruption may occur due to the effect of plasma-generated oxidizing species on membrane components [19]. Other researchers stated that membrane fracture after plasma application could be due to charge accumulation on the membrane's outer surface. This event occurs when the outward electrostatic forces exceed the strength and tensile force of the cell membrane [4]. The cell wall is initially under pressure and is weakened by the oxidation interactions of active species until the electrostatic force exceeds the tensile forces and causes a decrease in tensile strength. So this leads to wall fracture [25]. In 2013, Fernandez et al. studied the effect of cold plasma on the inactivation of *Salmonella Typhimurium* using nitrogen gas. Also, the surfaces of lettuce, strawberry, and potato samples were contaminated with *Salmonella,* and the effect of cold plasma on the disinfection of these products was also evaluated. In favorable conditions, after applying plasma for 2 minutes, the number of *Salmonella* bacteria decreased by 2.71 logarithmic cycles. While to reduce the same bacteria on the surfaces of lettuce, strawberry, and potato, 0.94, 1.76, and 2.71 logarithmic cycles were needed for 15 minutes of plasma application, respectively. So that the time required to inactivate *Salmonella* in the environment was 2.71 logarithmic cycles, it was much shorter than the time needed to inactivate Salmonella on the surface of lettuce. Therefore, their decontamination is essential to increase shelf life. It can preserve their physicochemical and sensory characteristics.These results show that the efficiency of cold plasma treatment is closely related to the surface characteristics of the product. The research showed that plasma affects food components, including water, fat, protein, carbohydrate, and phenolic compounds. It should be noted that plasma reactive species do not penetrate the food. As a result, the change occurs only at the level of food components. The cytoplasmic membrane represents the boundary between the inner compartment of the cell and the outer environment. Consequently, the first target of the species formed in the plasma is the cytoplasmic membrane. Structural strength or specific function in the cytoplasmic membrane can affect plasma function [31]. During treatment with non-thermal plasma by ozone gas, hydroxyl radicals, hydrogen peroxide, active oxygen, nitrogen oxides, and UV rays are produced. The species affected different molecule parts, such as DNA, proteins, and polysaccharides. So that each species will destroy other parts of the microorganism;

for example, UV rays can change the DNA of microorganisms and stop cell reproduction. The double bonds of unsaturated fatty acids are particularly vulnerable to ozone. Membrane lipids are significantly affected by Reactive Oxygen Species (ROS) and are bombarded by this powerful oxidant. The cell wall will be torn due to the accumulation of charge on the outer surface. The contribution of each of the above mechanisms in the inactivation of microorganisms depends on the plasma characteristics and the type of microorganism [19,25].

Misra et al. also investigated the effect of dielectric plasma on strawberry samples in packages with nitrogen and oxygen gas. In this experiment, the strawberries' initial total count and the initial mold and yeast counts were 4.99 Log cfu/g and 4.96 Log cfu/g, respectively. After plasma application, the total count of mold and yeast for samples packed with nitrogen gas equals3.7 Log cfu/g and 3.3 Log cfu/g, respectively, and for pieces filled with oxygen gas, 3.1 Log cfu/g and 3.4 Log cfu/g were reported [21]. In another study, Misra et al. reduced the total count and mold and yeast count by 2.4 and 3.3 logarithmic cycles by using dielectric barrier discharge plasma in packing strawberries with air gas. The performance of nitrogen gas and oxygen in reducing total microorganisms was better than air gas, but in reducing mold and yeast, all three gases had the same performance [22].

Evaluation of Texture Hardness Changes

The texture is one of the most important quality parameters in evaluating shelf life and consumer acceptability. The softening of the surface of fruits and vegetables occurs due to the destruction of the cell wall due to the activity of decomposing enzymes. In addition, fungal contamination also causes texture aging and breaking of the cell wall, thus reducing the hardness of the texture. As a result, this study evaluated the hardness of cucumber tissue immediately after plasma application. (Figure 2) shows the results of the effect of device power and duration of plasma application on cucumber texture hardness. The impact of the time of plasma application on tissue hardness was insignificant. The results showed that the structure of the tissue remains intact after the application of plasma, and plasma does not have a destructive effect on the design of the tissue. The samples treated with plasma had a better texture than the control sample during storage. The best surface was observed in the models affected by higher-power plasma for a more extended period. Misra et al. reported similar results using atmospheric pressure dielectric barrier discharge plasma using air gas on strawberry samples [22].

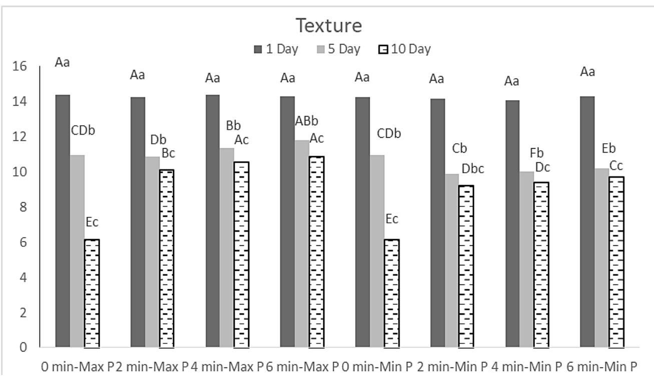

Figure 2: The comparison of the average power of the plasma device and duration of plasma application on the firmness of cucumber tissue during the storage period. (Lowercase letters refer to comparing one treatment on different days, and upper-case letters refer to comparing treatments in one day). These means with at least one similar letter do not have a significant difference at the probability level of 0.05 with Duncan's test.

Ramazzina et al. investigated the effect of dielectric barrier discharge plasma on kiwi slices' physical and chemical parameters. This study measured the tissue of control and treated samples after plasma application for 20 and 40 minutes. No significant difference was observed between the control group and the group treated with plasma [29]. Tappi et al. also subjected cantaloupe slices to dielectric barrier discharge plasma for 30 and 60 minutes and measured tissue hardness. This study reported no significant difference between the control and plasma-treated groups [34]. The overall evaluation of the results showed that the samples treated with plasma had more muscular tissue than the control group. This issue can be due to the reduction of surface microorganisms of samples treated with plasma, increasing the shelf life of cucumbers. Abbaszadeh and Bagheri reported that the tissue strength of plasma-treated samples at the end of the fourth day of storage was higher than the tissue strength of the control samples [2].

Evaluation Results of Color Indicators

Few studies have evaluated physical and chemical properties after plasma processing. While plasma technology uses charged particles to interact with food, chemical reactions such as vitamin E and C degradation have not been thoroughly studied. Since the plasma treatment is applied only on the product's surface and most of the reactions take place on the surface, the physical and appearance evaluation to study the effect of plasma can be a good criterion for this purpose. Any change in color, texture, or aroma can indicate a chemical reaction in the product. Color is one of the critical factors in the quality evaluation of cucumber, and it plays a crucial role in its selection and acceptance and may even affect the taste.

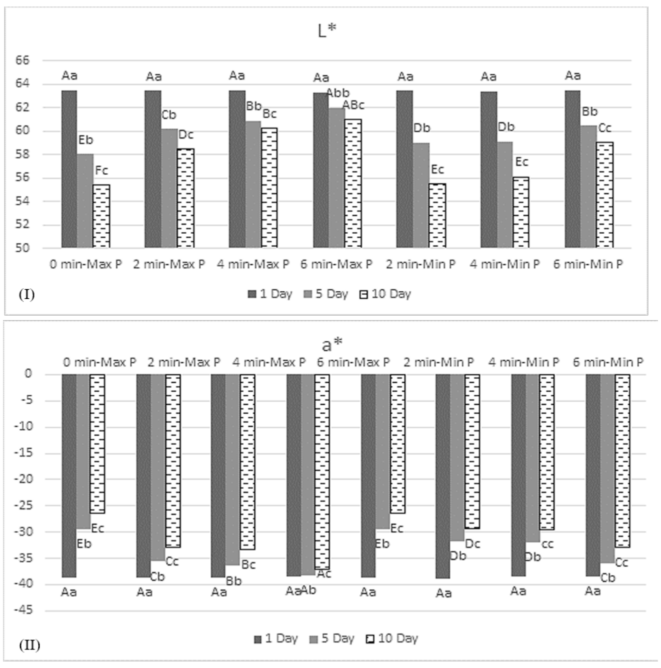

Figure 3: Comparing the average effect of the power of the plasma device and the duration of plasma application on the factors: **(I)** L*, **(II)** a*, and **(III)** b* related to the color during the storage period. (Lowercase letters refer to comparing one treatment on different days, and upper-case letters refer to comparing treatments in one day). These means with at least one similar letter do not have a significant difference at the probability level of 0.05 with Duncan's test.

The results showed no significant difference between the color of the control samples and those treated with plasma immediately after plasma application. Therefore, plasma does not affect the color indices of the models. Also, comparing the average color indices showed that the cucumbers on the first day were higher than all three-color indices. With the increase in storage time, the value of all three indicators decreased significantly ($P < 0.05$) (Figure 3).

Bermúdez-Aguirre et al. [3] studied the effect of atmospheric pressure jet discharge plasma on the color indices of tomato, lettuce, and carrot products. The results showed an increase in the value of a* (red) after 7 and 10 minutes of plasma application for tomato and lettuce products. Of course, this increase was not statistically significant. There were no substantial changes in the color indices for the carrot product before and after the plasma application. In another study in 2014, Misra et al. reported changes in the color indices of strawberries treated with air plasma, but these changes were minimal compared to the control sample. As a result, plasma does not have a destructive effect on the product's color. Smeu et al. [32] investigated the quality characteristics of lettuce during storage using atmospheric pressure jet discharge plasma. Significant changes in color indices were reported on the fourth day of storage compared to the control samples. The most important difference was observed in a* index of the pieces. Also, L* index showed uniform changes during maintenance.

Sensory Evaluation Results of Cucumber Samples

After plasma application, the treated and control samples were subjected to sensory evaluation. The examples were provided to the evaluators. Different treatments were compared in terms of color, aroma, taste, texture, and overall acceptance. The best sample was assigned a rank of 5, and the worst was given a level of 1.Based on the results, the sensory characteristics of the pieces did not show any changes after plasma application. During the storage period, samples treated with plasma better maintained their sensory characteristics due to less microbial load and reduced spoilage. Matan et al. [20] studied the effect of atmospheric pressure radio discharge plasma on pitaya fruit quality characteristics. The sensory evaluation results showed no significant difference between the sensory characteristics of the samples treated with plasma and the control group. Yong et al. [36] significantly reduced the bacteria on the cheese surface within 10 minutes of plasma application using a dielectric barrier discharge plasma system. The results showed a significant decrease in the characteristics of aroma, taste, and overall acceptance of the samples treated for 5 and 10 minutes of plasma application compared to the control sample. Lin et al. [18] also discussed the effect of jet discharge plasma on the sensory characteristics of egg yolk and white egg samples. The results showed that these characteristics showed a significant decrease for the egg yolk, while no significant difference was observed for the egg white between the sensory characteristics. They reported the cause of these changes to fat oxidation by free radicals formed in the plasma. According to the results of the conducted research, it can be concluded that the change in sensory characteristics by applying atmospheric pressure plasma probably depends on the type of food. For example, the oxidation of fats by free radicals formed in the plasma causes changes in the sensory characteristics of some foods.

Conclusion

They consider that products such as cucumber have a short life after harvest and are constantly exposed to microbial contamination. Therefore, their decontamination is essential to increase their shelf life while maintaining their physicochemical and sensory characteristics. In this study, the effect of cold plasma on increasing shelf life and physicochemical, sensory, and microbiological aspects of cucumber (Cucumis sativus var. Negin) during ten days of storage at room temperature were investigated.

The results showed that plasma application reduced the total count by 2.59 logarithmic cycles and a profile of mold and yeast by 1.388 logarithmic cycles compared to the control sample. The most significant decrease in the microbial profile was observed in the most extended duration of plasma application (6 minutes) and the highest power of the device (17 volts and 2.66 amperes). The color indices, texture hardness, and sensory evaluation of samples treated with cold plasma were not significantly different from the control sample. Non-thermal plasma is known as an efficient method for the inactivation of various microorganisms. This method has advantages such as no waste production, low energy consumption, low cost, and preservation of nutrients. Therefore, it can be a suitable alternative to other chemical methods (such as the chlorine process) and physical methods (such as high-pressure processes, electric pulses, and ionizing radiation) used for pasteurization and sterilization of food products.

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Conflicts of Interest

The authors declare that there are no conflicts of interest.

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