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Investigating the Effect of Cold Plasma on some Chemical Properties of Date Fruits (Phoenix dactylifera L.)

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ABSTRACT

In order to investigate the effect of atmospheric cold plasma on some chemical characteristics of date fruits, Mazafati variety, in storage conditions, cold plasma was applied to the samples using a sliding rotary arc discharge device. The independent variables in this study were plasma application duration in 5 levels (30, 60, 120, 180, and 240 s) and different gases in 3 types (air, argon and oxygen). Then, some chemical properties including weight loss percentage, pH and acidity, total soluble solids, total phenolic compounds, and reducing sugar were measured. The experiments were conducted in the form of a factorial design based on complete randomized design. The results showed a significant decrease in the weight of the samples treated with plasma compared to the control samples, during the storage period. The effect of cold plasma on Mazafati date fruits showed a decrease in pH, and an increase in total soluble solids during the storage period. The average value of total phenolic compounds was the lowest value for the samples treated with oxygen gas (2182 mg/kg) and the highest value for the samples treated with argon gas (4007 mg/kg) with plasma treatment time of 240 s. The values of reducing sugar were 38.76 % at the lowest and 65.54 % at the highest amounts, for the control and the samples treated with air with a plasma treatment time of 240 s, respectively.

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INTRODUCTION

Date is an important and strategic agricultural product of Iran. Reducing the microbial contamination of agricultural products, especially products with high economic value, is always one of the issues in which the use of radiation technology has been a suitable solution and has received attention in recent years. Although different varieties of dates have different chemical compositions, it is important to investigate the changes in the chemical composition of this fruit during the processing stages, including grading, storage, etc (Edalatian and Fazlara, 2008).

New methods of food preservation and decontamination without losing product quality have attracted a lot of attention. So far, several methods have been used to increase the shelf life of agricultural products, which can be classified into two categories: thermal methods and non-thermal methods (Ahmadnia et al., 2021).

Using the cold atmospheric plasma method as a non-thermal method is one of the emerging and promising technologies. Cold plasma, whose temperature is close to atmosphere temperature, is made up of energetic gas molecules, charged particles in the form of positive and negative ions, free radicals, electrons, photons and it can be used to inactivate pathogens on the surfaces of food. These effects are caused by the interaction of active charged particles, radicals and neutral particles that exist in plasma. One of the interesting features of plasma production systems is the possibility of choosing a gas or a combination of different gases. Often, noble gases are used to produce plasma, although they lead to an increase in cost. Ambient air can be used to reduce costs (Misra et al., 2011).

Dielectric barrier discharge method is the most common method to produce cold plasma at atmospheric pressure. In this method, two electrodes are used, at least one of them covered with dielectric. The distance between two electrodes is usually about a few millimeters (Salge, 1996). Considering that plasma has a high ability to inactivate microorganisms in food and requires relatively simple equipment, therefore, using this technology in industrial scale is definitely cost-effective.

The amount of total sugar in Mazafati date is reported to be around 62-70%, a small portion is sucrose (1-2%) and most of it is reducing sugars (60-68%). Microorganisms in dates will grow and develop according to initial moisture, pH and initial microbial load. In this way, the higher the initial moisture and initial microbial load and the closer the pH is to neutral, the higher the growth of microorganisms and the higher final microbial load is performed (Edalatian and Fazlara, 2008).

In a research, the effect of cold plasma treatment on the quality characteristics of grapes was investigated. Fakhri grapes were kept at 4˚C after plasma treatment for 0, 10, 20, and 40 seconds, and chemical properties (soluble solids, pH, and acidity) were evaluated for 35 days with a time interval of 7 days. Based on the obtained results, there was no significant difference in titratable acidity, soluble solids and pH in any of the treated samples compared to the control sample. Therefore, due to the significant reduction in the level of contamination and no change in the chemical properties of grapes, this method was introduced as an efficient method for controlling of grape waste (Khalaj et al., 2019).

In research by Misra et al. (2014), they studied the effect of cold plasma with dielectric barrier discharge on the chemical properties of strawberry samples packed with nitrogen and oxygen gas. The results showed that plasma treatment had no significant effect on the chemical characteristics of strawberry samples.

In research conducted on date fruit, the surface discharge of the dielectric barrier was used to prevent the growth of Aspergillus Niger. In this research, the effect of cold plasma on chemical content and levels of hydroxymethyl furfural (HMF) of some date varieties was evaluated. The results of this study showed an increase in phenolic content, an increase in antioxidant activity, and a decrease in the activity of Aspergillus Niger in the treated samples compared to the control (Lotfy et al., 2022).

Although cold plasma treatment on fruits and vegetables can help to preserve favorable properties, at the same time, long-time treatment can lead to adverse chemical effects. To fully

evaluate this method, food quality issues such as changes in nutrients, color, textural quality, and other chemical changes must be considered. Therefore, the aim of this study is to obtain the optimal time of cold plasma treatment and to determine the amount of required gas for plasma treatment, taking into account the quality of the product during the storage period and also to investigate some chemical characteristics of the Mazafati date fruit after plasma treatment. The goal of chemical control of dates is to increase the storage time, reduce the amount of waste and maintain the quality of its nutrients. having complete information about the chemical compositions of dates, is important in its grading, storage and processing stages. Finally, the optical emission spectroscopy method is used to investigate the presence of spectra related to UV, hydroxyl and nitrogen molecule as mechanisms of inactivation of microorganisms.

MATERIALS AND METHODS

In order to investigate the effect of cold plasma on some physical and chemical properties of Mazafati dates, date samples were taken from the orchards of Rostam Abad district of Narmashir city (Bam, Kerman, Iran) in the Rutab stage. To prevent hand interference samples were harvested with the cluster and under controlled temperature conditions and in the shortest possible time, it was transferred to Butia Company (Kerman, Iran). The selection of samples was done manually and under laboratory conditions. Date samples were selected in the same conditions in terms of shape, color, size and freshness, and the samples were subjected to plasma treatment in a sterile environment. The plasma reactor used for plasma generation was a sliding rotary arc type with arc discharge, capable of working at atmospheric temperature, and the gas used for it was air, argon, and oxygen. The design and construction of this prototype was done by Butia compony researchers. Figure 1 indicates the schematic of sliding rotary arc generator used in this research.

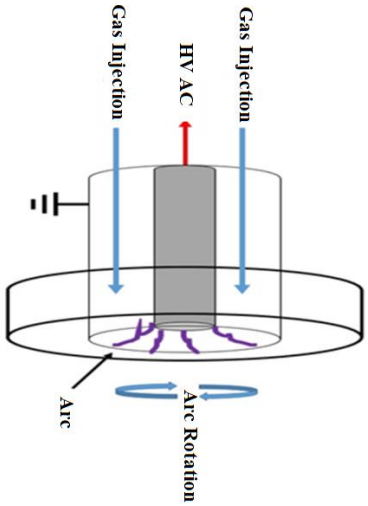

Figure 1. (a) Schematic of the sliding rotary arc plasma generator

The power source of this reactor was an AC type (High voltage) with a reactor voltage of 6.4 kV and a reactor frequency of 50 kHz with the ability to work at atmospheric temperature. Plasma treatment based on changing the duration of plasma treatment to Mazafati date samples at 5 levels (30, 60, 120, 180 and 240 s) and the type of gas used at three types (air, argon and oxygen gases) with a flow rate of 0.5 L/s applied. The treated samples in sterile containers with lids were transferred to the laboratory of the food industry science and engineering department of the Shahid Bahonar University of Kerman to perform experiments.

The process of changing the quality characteristics of dates during the storage period after plasma treatment and at ambient temperature was evaluated. Before plasma treatment of date samples, the surrounding area of the machine and the location of the sample and the containers used were disinfected using 70% ethanol and cold plasma, and plasma treatment was performed in a completely sterile environment. In order to ensure the application of plasma treatment to all the surfaces of Mazafati date samples and due to the absence and installation of a rotation system for the location of the sample, the rotation of the samples was done manually and according to the time of plasma treatment. A distance of 2 cm from the place of plasma creation to the location of the sample was set and taken into consideration (Figure 2).

Figure 2. The sample plasma treatment by the sliding rotary arc reactor

Weight loss measurement

Weight changes are among the effective quality characteristics of fresh fruits and vegetables. To measure weight changes, Mazafati date samples were placed in sterile containers after plasma treatment. Then, the weight of the samples was measured 9 days after plasma treatment with a digital scale with an accuracy of 0.001 grams and the samples were kept at ambient temperature, and finally, the weight loss percentage of the samples was calculated through equation 1. In this way, the weight loss percentage during storage in different treatments was compared and the weight loss percentage of the samples (W_R) compared to the initial weight (W_i) was calculated.

$$W_R = \frac{W_i - W_2}{W_i} \times 100 \quad (1)$$

Measurement of the content of phenolic compounds

To measure the amount of total phenolic compounds in the fruit, Folin Ciocalteu reagent was used (combining 3 ml of reagent and 27 ml of distilled water). The absorbance of the reaction mixture was read at a wavelength of 750 nm using a spectrophotometer (UNICO, China, 2002) (Zarbakhsh & Rastgar, 2018). In order to measure total phenolic compounds, 0.25 grams of Mazafati date fruit flesh was extracted from each replicate in 4.9 ml of solvent (80 ml of methanol and 20 ml of distilled water). After stirring with a shaker (KS 260 BASIK, IKA, Germany) for 1 hour, it was placed in a model centrifuge (Sigma, 3-30K, Germany) at 4000g for 10 minutes to settle the suspended material. In the next step, the clear liquid that was placed on top was used to measure the content of phenolic compounds and the amount of 100 microliters of obtained extract was mixed with 0.75 ml Folin Ciocaltio reagent (diluted 10 times with distilled water) using a sampler. After 5 minutes at room temperature, 0.75 ml of sodium bicarbonate was added to the mixture. After staying for 30 minutes at room temperature and in a dark place, the absorbance was measured in three repetitions by a spectrophotometer at a wavelength of 750 nm and the data was recorded. Gallic acid was used to prepare the standard curve and the results were expressed as milligrams of gallic acid per gram of fresh fruit weight (Nadernejad et al., 2013).

Reducing sugar

To measure the reducing sugar, 0.5 grams of Mazafati date fruit flesh was added for each replicate to 9.5 mL of distilled water. After mixing with a shaker (KS 260 basik, IKA, Germany), for 15 min. Then, using a sampler, mix 100 microliters of the prepared extract with 4.9 mL of distilled water, and finally mix 600 microliters of the extract with 800 microliters of the solution (reagent) and it was placed at a temperature of 80 degrees Celsius for 5 to 10 minutes and immediately, the amount of absorption was measured in three repetitions by a spectrophotometer at a wavelength of 575 nm and the data was recorded.

Acidity and pH

The acidity of dates was measured by titration with 0.1 normal sodium hydroxide (Ayobi, 2017). To measure titratable acidity and pH, after separating the date kernel, 2.5 grams of Mazafati date flesh from each replicate was extracted in 25 ml of distilled water. Titratable acidity based on milligrams of citric acid per 100 grams of fruit tissue was performed by titrating date fruit extract with 0.1 sodium bicarbonate solution and the pH of the extract was measured with a digital pH meter (Zag Chemistry, Iran) (Garcia et al., 2004). Most garden products have large amounts of organic acids, which are effective in the quality of these products, especially fruits. The most

important organic acids of date fruit are malic acid and citric acid (Mortazavi et al., 2006).

Total soluble solids (Brix index)

The amount of Brix was measured with a desktop refractometer (model 2WAJ, Optika, Italy) and the method recommended in the national standard of Iran 5075. To measure the amount of Brix, a drop of the extract was placed on the refractometer's prism and spread uniformly on the surface of the prism by observing the non-contact of the glass spatula and then the concentration was read at 20 degrees Celsius. This feature was done in 3 repetitions for each of the treatments. Sugars are the main soluble solids in fruits. Organic acids, amino acids, vitamins, and minerals are other soluble solids in fruits, whose amount is less than that of sugars (Ayoubi, 2017).

Spectroscopy

In order to investigate the presence or absence of UV irradiation and hydroxyl (OH) accompanied by cold atmospheric plasma, spectroscopy was performed. Spectroscopy was performed using Spectra Suite software and a USB2000 spectrometer manufactured by Ocean Optic. This spectrometer had a linear CCD and a spectral resolution of 0.035 nm, and the optical fiber collector was placed at a distance of 3 cm from the plasma column. This spectrometer stores a complete spectrum every millisecond between wavelengths of 200-1100 nm. In this research, the optical emission spectrometry (OES) method based on the analysis of the light resulting from the scattering of plasma particles (including neutral atoms, ions and molecules) was used. In this method, when the light hits the optical fiber collector, a graph is obtained based on intensity in terms of wavelength. In fact, its light intensity can be used to describe the excited species in each wavelength. In this method, the light resulting from the allowed electron displacements between energy levels is measured.

Statistical method

In this research, the duration of plasma application in 5 levels (30, 60, 120, 180 and 240 seconds) and the type of gas used in 3 levels (air gas, argon gas and oxygen gas) were considered as independent variables. SAS software was used for statistical analysis of data. The tests were conducted in the form of factorial design based on complete randomness. A comparison of sample averages was done based on Duncan's multi-range tests. The significant difference between the means was also analyzed by MSTAT-C software and EXCEL2016 software was used to draw graphs.

RESULTS AND DISCUSSION

In this research, the analysis of the results related to the effects of plasma application duration treatments and the type of gas used after plasma application with the atmospheric pressure arc discharge plasma reactor on some physical and chemical properties of the product such as weight loss percentage, pH, acidity, Soluble solids, total phenolic compounds and reducing sugar are provided and then the spectroscopy results are examined.

Weight loss is one of the important indicators during the storage period of fruits and vegetables, and it is important from the economic point of view, as well as the effect on the quality characteristics. Based on the obtained results, the interaction effect of plasma treatment duration and gas type on the weight loss percentage of date samples was significant at the level of 1%.

Plasma treatment significantly reduced weight loss in date samples so that the lowest average weight loss percentage was observed in samples treated with oxygen and argon gas plasma, and the highest weight loss (14.73%) was related to the sample treated with air gas. According to the effect of plasma treatment time on the investigated factor, the highest average weight loss percentage was seen in the sample with plasma treatment time of 30 seconds. The weight loss percentage showed an increasing trend during the storage period and the highest average weight loss percentage was recorded at the end of the storage period. Figure 3 shows the weight loss percentage of Mazafati date samples for different treatments during the storage period. Weight loss in dates is related to the decrease in moisture content under the influence of temperature during storage. The lower the amount of oxygen and

temperature of the environment and the higher the concentration of carbon dioxide, the lower the weight loss of the product will be (Qu et al., 2022).

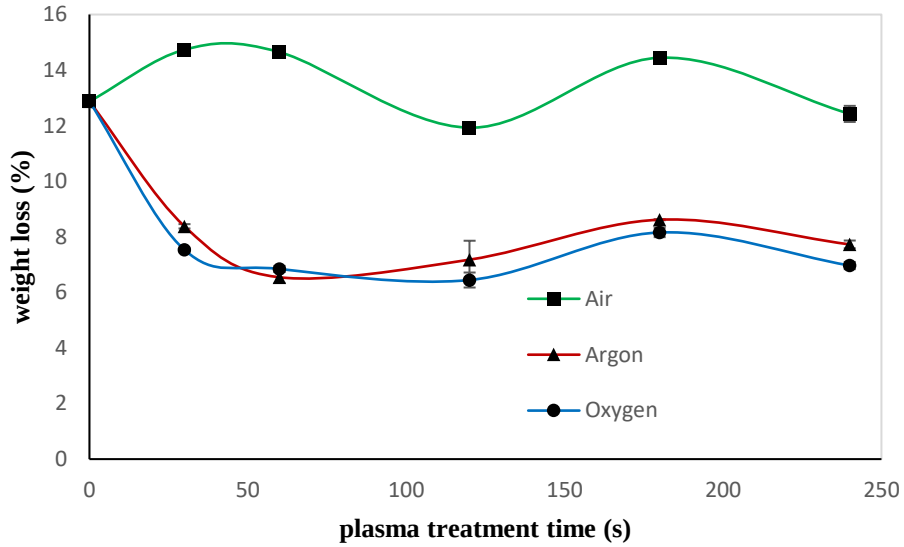

Figure 3. The weight loss percentage in date samples

Statistical analysis showed that the effect of gas type and plasma treatment time on pH value was significant at the level of 1%. The highest average pH was observed in the air-treated plasma sample (7.10). Figure 4 shows the pH diagram in the samples of Mazafati dates plasma treated with different gases and the control samples during storage. As can be seen, the effect of cold plasma on the Mazafati date product shows a decrease in pH value during the storage period. A decrease in pH with increasing storage time and also due to increasing the amount of organic acids and acidity is not far from expected.

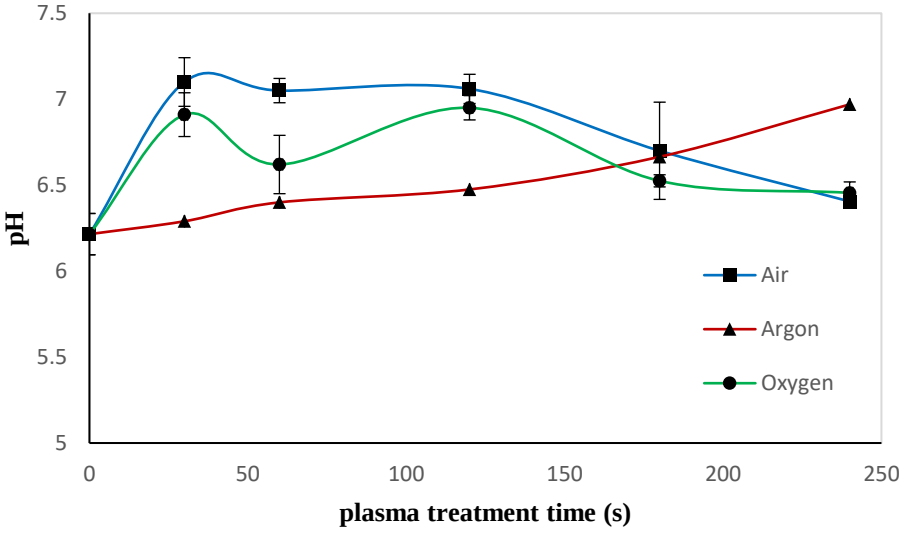

Figure 4. pH in Mazafati date samples

Considering that most fruits have organic acids, this factor is effective in the quality of these products. Malic acid and citric acid are the most important organic acids in date fruit (Ayoubi,

2017). As a result, checking acidity in fruit is of particular importance. The results of statistical analysis showed that the interaction effect of plasma treatment time and gas type on the acidity level was significant at 1% level. The level of acidity in the control sample (0.189) was significantly higher than in the plasma-treated samples. The lowest average level of acidity was observed in the sample treated with oxygen gas (0.148 %). The plasma treatment time had a significant effect on the acidity of the samples and the highest average acidity level (0.193 %) was observed in the samples with 240 seconds of plasma treatment. Figure 5 shows the acidity diagram in Mazafati date fruit samples treated with plasma with different gases and control samples during storage. As can be seen, the effect of cold plasma on Mazafati date product shows an increase in acidity during the storage period and this increase in acidity may be due to the increase in the intensity of fermentation and the activity of microorganisms under the temperature conditions of the environment, the aeration of the environment and the creation of suitable conditions for the production of organic acids by aerobic microorganisms.

The results of the analysis of variance showed that the effect of gas type and duration of plasma treatment on total soluble solids was significant at the level of 1%. The highest average of total soluble solids was observed in the samples treated with air plasma (70.95) and the lowest average was observed in the control sample (61.20). Figure 6 shows the total soluble solids (Brix) in the samples of Mazafati date fruit plasma treated with different gases and the control samples during storage. As can be seen, the effect of cold plasma on the product shows an increase in the amount of total soluble solids during the storage period. In fact, an increase in temperature or exposure to ambient air causes more water to escape from the fruit and an increase in the sap concentration. The results of Ayubi research (2017) indicated an increase in the concentration of soluble solids in the dates with an increase in temperature and storage time.

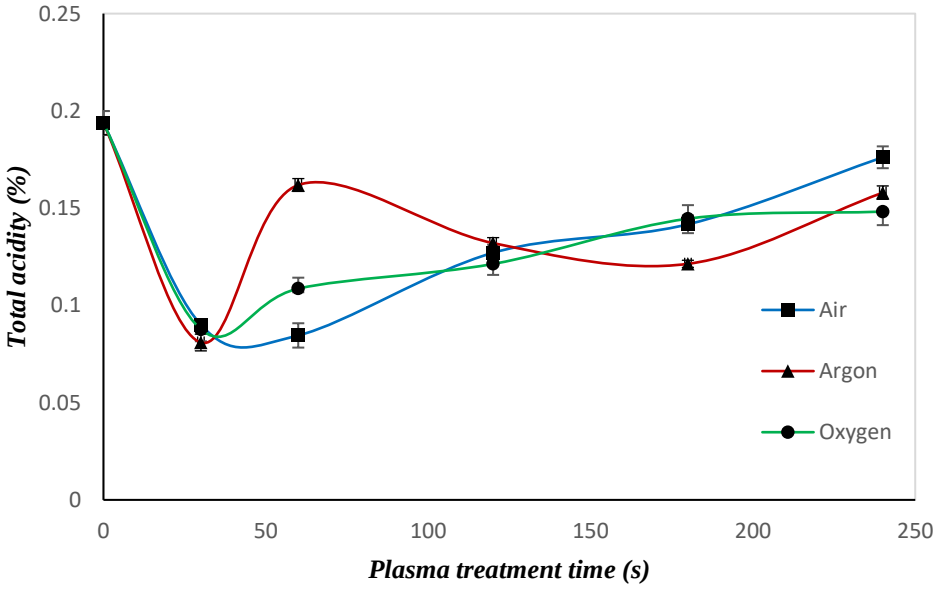

Figure 5. Acidity in Mazafati date samples

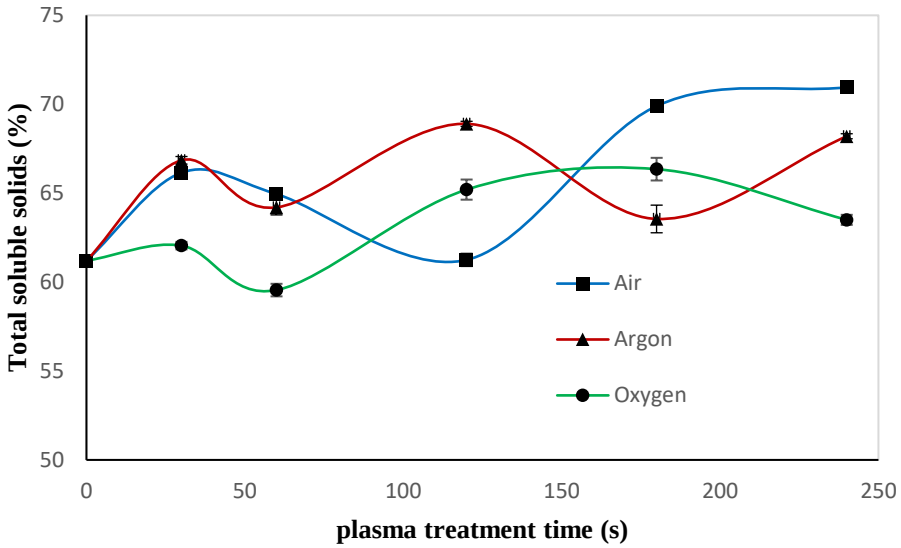

Figure 6. The amount of total soluble solids in date samples

An important part of the nutritional value of fruits is related to compounds with antioxidant properties. The results of the analysis of variance showed a significant mutual effect of gas type and plasma treatment time on total phenolic compounds at 1% level. The highest average amount of total phenolic compounds was observed in the argon-treated sample (4007 mg/kg FW) Also, the average amount of total phenolic compounds in oxygen-treated samples with a plasma treatment time of 240 seconds. (2182 mg GA/kg FW) was lower than other treatments. Figure 7 shows the graph of the amount of total phenolic compounds in the samples of Mazafati dates plasma treated with different gases and the control samples. As can be seen, the effect of cold plasma on the Mazafati date product shows a decrease in the content of phenolic compounds and antioxidant compounds in the plasma-treated samples compared to the control sample. The reduction of phenolic compounds in some plasma treatments may be due to the possibility of degradation of phenolic compounds under plasma treatment (Garofulić et al., 2015).

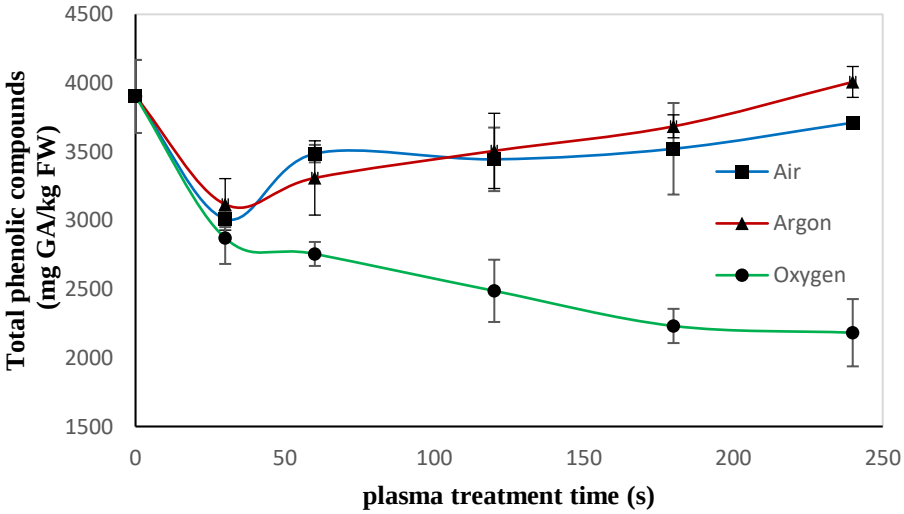

Figure 7. The amount of total phenolic compounds in date samples

The results of the analysis of variance showed that the interaction effect of gas type and duration of plasma treatment on the amount of reducing sugar is significant at 1% level. The highest average amount of reducing sugar in the air-treated plasma sample (65.54 %) and the lowest average amount of reducing sugar in the control samples (38.76 %) were observed. Also, the difference between the control treatment and plasma-treated samples was significant ($p<0.05$). The highest amount of reducing sugar was observed in the samples that were exposed to plasma for 30 seconds and the lowest amount was observed in the samples that were exposed to radiation for 240 seconds. Figure 8 shows the graph of the amount of reducing sugar in the samples of Mazafati date fruit treated with plasma with different gases and the control samples. The results of investigating the effect of cold plasma treatment on pitaya fruit (dragon fruit) showed that the content of glucose and fructose gradually decreased under plasma treatment. Cold plasma increased the consumption of two sugars, glucose and fructose, and lowered their levels compared to the control sample. The accumulation of phenols, which is caused by the stress applied to the fruit, inevitably causes more consumption of glucose and fructose, which are the predominant sugars in fruits (Li et al., 2019).

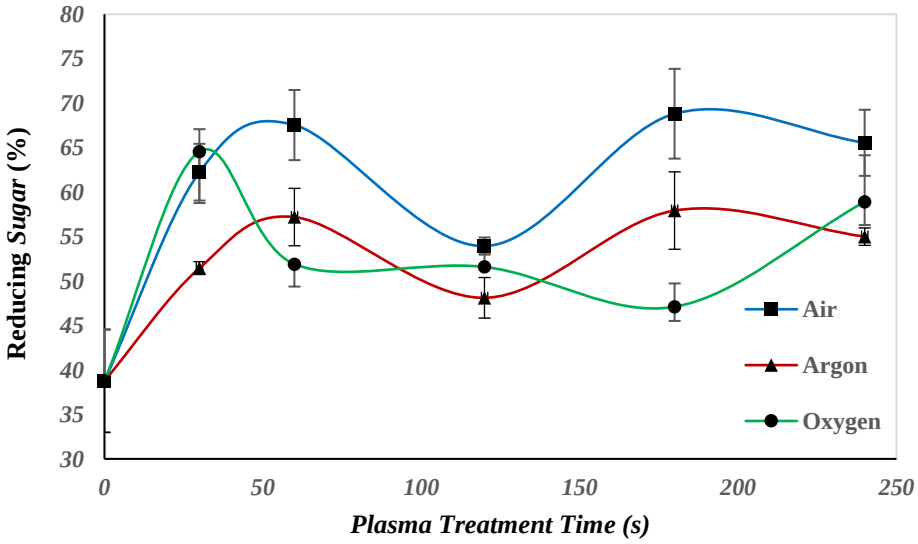

Figure 8. The amount of reducing sugar in date samples.

In figures 9 to 11, the optical emission spectra corresponding to three states are presented, in which air, argon, and oxygen are introduced into the reactor chamber at atmospheric pressure, respectively. As can be seen in Figure 9, the optical emission spectrum of the plasma resulting from the entry of air at atmospheric pressure with a sliding rotation arc light source, the spectrum related to UV, hydroxyl (OH) and nitrogen molecule (N_2) was produced. Among the inactivation mechanisms of cold plasma is the production of UV rays in the wavelength of 200-300 nm, which causes damage to the DNA of microorganisms. UV rays with a wavelength $\leq$ 257 can break C-H and C-C bonds and cause the inactivation of microorganisms (Reineke et al., 2015). In fact, UV rays lead to the damage of thymine and cytosine in the DNA strand, thus preventing the proliferation and replication of bacteria.

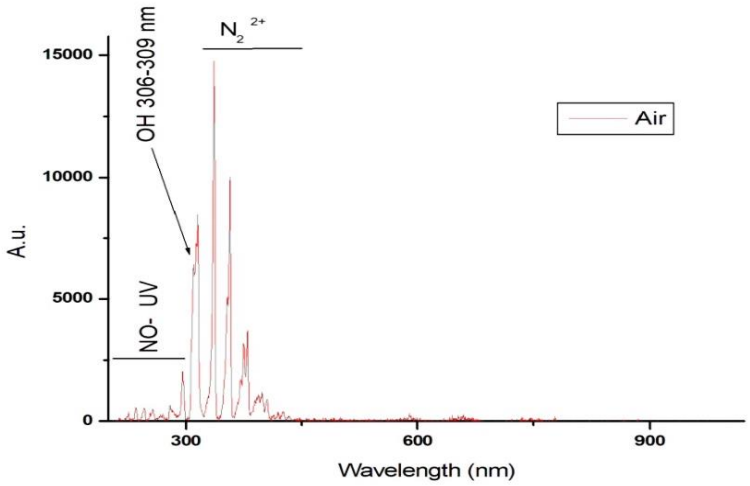

Figure 9. The optical emission spectrum of the plasma resulting from the entry of air gas at atmospheric pressure into the sliding arc reactor

As can be seen in Figure 10, in the optical emission spectrum of the plasma resulting from the entry of argon at atmospheric pressure into the sliding arc reactor, the spectrum related to UV is very small, but hydroxyl (OH), nitrogen molecule (N_2) as well as argon (Ar) and oxygen (O) have been produced. Hydroxyl radical is a very reactive species that causes serious damage to biological molecules and its presence has been reported in many types of plasma. These radicals have a high speed of interaction with any molecule present in living cells. The high oxidation potential of OH (2.86 V) shows its strong oxidizing property. OH has a diffusion distance of the order of nanometers, so it can interact with the components of the cell membrane. One of the targets of active species is DNA, and hydroxyl radicals are considered one of the most important factors of DNA damage due to their high activity.

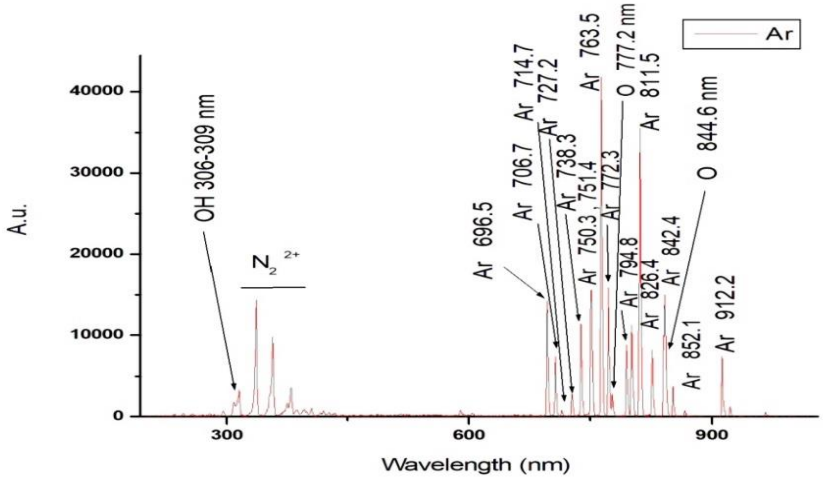

Figure 10. Optical emission spectrum of the plasma resulting from the entry of argon gas at atmospheric pressure into the sliding arc reactor

Also, based on Figure 11, it can be seen that in the light emission spectrum resulting from the introduction of oxygen at atmospheric pressure into the sliding arc reactor, there is a spectrum

related to UV, hydroxyl (OH), oxygen atom (O) and nitrogen molecule (N_2). Species produced in the process of non-thermal plasma treatment, such as ozone, hydroxyl radicals, Hydrogen peroxide, reactive oxygen, and nitrogen oxides, as well as UV rays, affect different parts of molecules, such as DNA, protein and polysaccharides. So that each species will destroy different parts of microorganisms. The double bonds of unsaturated fatty acids are particularly vulnerable to ozone attack. Membrane lipids are significantly affected by reactive oxygen species (ROS) and are bombarded by this powerful oxidant. The contribution of each of the above mechanisms in the inactivation of microorganisms depends on the characteristics of plasma and the type of microorganism (AhmadNia et al., 2021).

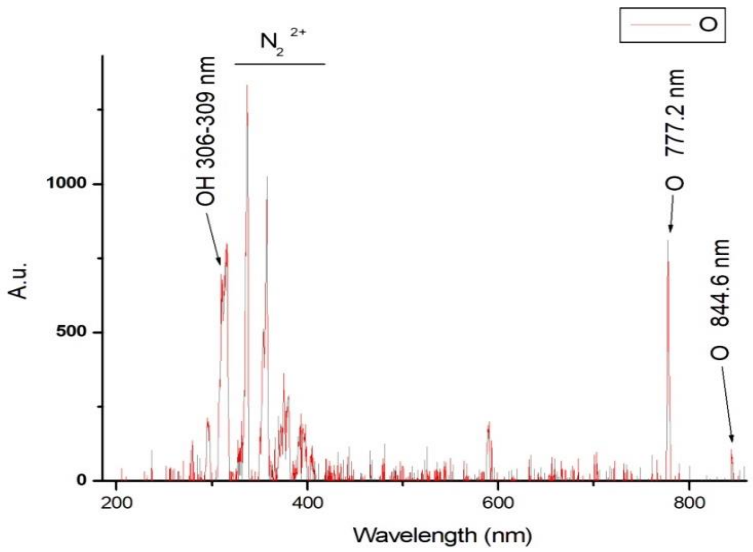

Figure 11. Optical emission spectrum of the plasma resulting from the entry of oxygen gas at atmospheric pressure into the sliding arc reactor

CONCLUSION

One of the concerns of using the cold plasma method is affecting the quality of the food therefore, to fully evaluate this method, food quality issues such as changes in nutrients, color, toxic residues and other chemical changes should be considered. Also, before using the plasma method directly in the food industry, research on the price of the process for large amounts of food as well as the health of the gases should be considered. In this research, some chemical properties including parameters of total phenolic compounds, reducing sugar, acidity, pH and soluble solids were evaluated. In order to be successful in exporting dates, different methods should be presented and used in order to reduce waste and cost-effective solutions to increase shelf life in order to increase investment in production and export in addition to preventing date spoilage and preserving the appearance of dates.

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