

# Evaluation of the Nutritional and Biochemical Variation in Pistachios Exposed to Dielectric Barrier Discharge Plasma

Mahdiyeh Bakhtiyari-Ramezani<sup>a,\*</sup>, Fatemeh Amani<sup>b</sup>, Aboutorab Naeimabadi<sup>c</sup>

<sup>a</sup> Plasma Physics and Nuclear Fusion Research School, Nuclear Science and Technology Research Institute (NSTRI), Tehran, Iran

<sup>b</sup> Plasma Technology Development Company, Tehran, Iran

<sup>c</sup> Department of Energy Engineering and Physics, Amirkabir University of Technology, Tehran, Iran

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## ABSTRACT

Cold plasma processing systems have proven to be promising tools for reducing biological and chemical contaminants in the food industry. However, little is known about the effects of plasma technology on the biochemical characteristics of food, particularly nuts. Therefore, this study evaluated the potential adverse effects of cold plasma on the biochemical characteristics of pistachio in 5, 10, 20, and 40 min of treatment. The results indicated that compared to the control group, there were no significant changes in photosynthetic pigments, malondialdehyde, catalase, peroxide value, acidity value, moisture content, and sensory attributes of pistachio. However, a significant decrease in the linoleic acid (5 %) alongside an increase in the palmitic acid (8 %) for 40-minute plasma treatment was observed. The total carbohydrates and protein content of pistachios remained unchanged for the 10-minute plasma treatment, while prolonged treatment (20 and 40 min) significantly reduced these compounds compared to the control group, with protein levels decreasing by 12.29 % and 14.56 %, and carbohydrate levels decreasing by 22.03 % and 44.0 %, respectively. Therefore, plasma treatment time is a critical factor in nut processing. Subsequent research should improve device design and optimize treatment duration to regulate the risk and food safety concerns.

## 1. Introduction

Cold atmospheric plasma (CAP) is an innovative technology in the food industry. Plasma is the fourth state of matter, comprising physical and chemical constituents, and is defined as a quasi-neutral ionized gas. Cold plasma (CP) is a complex medium of reactive oxygen species (ROS) and reactive nitrogen species (RNS), including atomic oxygen (O), ozone (O<sub>3</sub>), hydroxyl radicals (•OH), and nitric oxide radicals (NO•). Apart from these, it mainly includes photons and ions, besides the free electrons with a net neutral charge that can readily interact with molecular species, whether in a ground or an excited state (Pankaj et al., 2018). A significant strength of CAP is that throughout the treatment process, the temperature is close to room temperature (Ganesan et al., 2021). CAP treatment is a promising technology for food preservation and the inactivation of microorganisms, including bacteria, fungi, and subsequent mycotoxin, yeast, bacterial endospores, and biofilms on food surfaces, especially different nuts, such as hazelnuts, peanuts, or pistachios (Ganesan et al., 2021; Shabbir et al., 2024; Usman et al., 2023; Zhao et al., 2023). The dielectric barrier discharge (DBD), plasma jet,

and corona discharge are CAP processing systems for food applications. Among these, the DBD system, with its attributes such as low capital investment, expandability, and almost effortless operation, has many applications in different industries, such as the food industry (Beyrer et al., 2020).

Among the various types of nuts, over the fifty years, global pistachio production has shown an upward trend due mainly to their taste qualities, nutritional values, and potential health-promoting elements such as protein, unsaturated fatty acids, minerals, vitamins, phytosterols, and polyphenols (Hojjati et al., 2015). Pistachio nut (*Pistacia vera* L.) belongs to the plant species from the botanical family Anacardiaceae, and it is widely recognized as one of the leading tree nuts. As reported by FAO statistics (2023), global pistachio production amounts to 1303,462 tons. The leading producers of this commodity from 2013 to 2023 are the United States, Iran, and Turkey, with average annual productions of 373,000 tons, 361,000 tons, and 156,000 tons, respectively. China, Syria, Greece, Madagascar, and Spain have been classified as minor producers of this particular product (FAO., 2023; Hassan et al., 2024). Iran is one of the world's largest producers and exporters of pistachios,

\* Corresponding author.

E-mail address: [mahdiyeh.bakhtiyari@gmail.com](mailto:mahdiyeh.bakhtiyari@gmail.com) (M. Bakhtiyari-Ramezani).

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and they play a central role in the country's economy (Hojjati et al., 2015), thus making research in this regard of considerable practical importance.

Numerous studies have been conducted regarding the effects of CAP on the sterilization of pistachios. Dasan et al. (2016) found that the atmospheric pressure fluidized bed plasma system effectively eradicated *Aspergillus flavus* and *Aspergillus parasiticus* on hazelnuts within 5 min. Sen et al. (2019) reported that treatment with low-pressure plasma at 100 W for 30 min resulted in a population reduction of *A. flavus* and *A. parasiticus* on hazelnuts of approximately 4 log. The study by Makari et al. (2021) demonstrated that increased plasma treatment time directly affected lowering the number of viable spores. Devi et al. (2017) reported that DBD plasma operating at 60 W can reduce >95 % of the decontamination of aflatoxins present on pistachios. Los et al. (2018) presented that DBD plasma processing applied to wheat and barley reduced bacterial counts; the highest reduction was achieved: 3.2 log<sub>10</sub> CFU/g of *Bacillus atrophaeus* and *Escherichia coli*, respectively.

Microbial degradation and inactivation in CP applications are affected by the duration of exposure, the particular gas utilized for disinfection and natural composition of the nuts. While longer exposure times are usually indicated by increased effectiveness in sterilization, problems arise as exposure may also adversely affect the bioactive compounds of the material being treated (Ganesan et al., 2021). The majority of published studies have primarily focused on the impacts of CP on the functional, morphological, and rheological characteristics of various plant-derived materials such as apple juice, kiwi, wheat (Chaple et al., 2020; Farias et al., 2020; Jaddu et al., 2024; Ramazzina et al., 2015); often overlooking in-depth analyses of specific nutritional components.

Research explicitly addressing the potential applications of CAP effects on the preservation of functional, nutritional, and organoleptic properties of pistachios is notably deficient, particularly with extended treatment. This leaves a substantial knowledge gap regarding its impact on nut oilseeds, specifically pistachios. In this regard, studies have suggested CP enhanced antioxidant bioaccessibility in pistachios but did not alter the total phenol content (Makari et al., 2021). The CP treatment could also change the lipid oxidation level, which may limit the quality and acceptability of the final products, and elevate the levels of certain beneficial nutrients, like  $\gamma$ -tocopherol, by a slight amount (Foligni et al., 2022). In peanuts, there were increases in solubility, emulsifying capacity, effective water holding capacity, and modifying peanut protein isolate solutions after CP treatment (Ji et al., 2018). CP treatment of groundnuts, specifically at 30 kV for 15 min, resulted in an increase in components such as protein content (28.96 %) and fat content (42.37 %) (Das et al., 2024). These findings suggest the potential of CAP to influence the nutritional and functional characteristics of nuts, but underscore the critical need for more focused research on its specific effects on pistachios, especially regarding quality and biochemical parameters. In order to fill the existing knowledge gaps regarding the nature of such complicated interactions, this study aimed to investigate the biochemical characteristics and nutritional quality of pistachios following plasma treatment with particular attention to treatment duration. This knowledge is crucial because the time of the plasma treatment should be treated very accurately in order to achieve an ideal balance between microbial reduction and its impact on the food quality. Specifically, the effects of longer treatment durations on their nutritional and organoleptic properties were examined. Furthermore, this research seeks to offer a detailed analysis of plasma's interaction with food components, thereby promoting the industrial application of CP technology.

## 2. Materials and methods

### 2.1. Cold atmospheric pressure plasma processing system

Dielectric barrier discharge plasma is generated by a high voltage applied between two metal electrodes, where a dielectric material

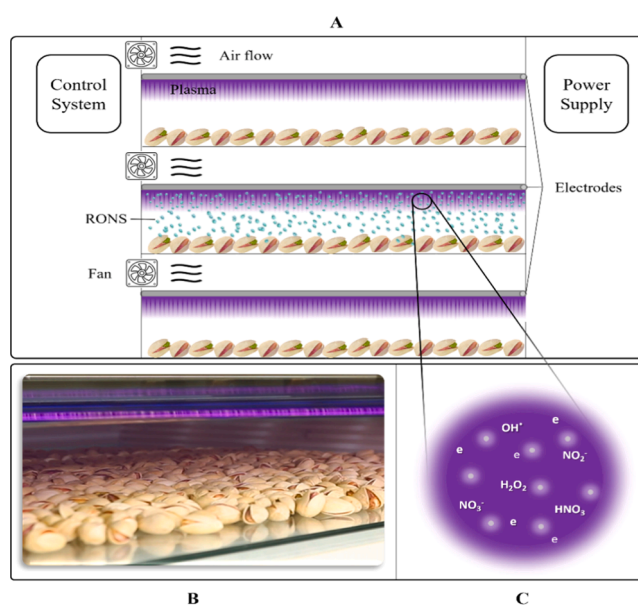
covers one or both electrodes. Fig. 1A illustrates the plasma source utilized for decontamination and sterilization, which is based on the dielectric barrier discharge and comprises four primary components: the main plasma reactor, fan, power supply, and control system. Fig. 1B shows the inside of a plasma reactor. The fans take ambient air into the plasma reactor. The produced species transferred to the placement area, as shown in Fig. 1C. A power supply provides the electrical power needed to create plasma, and the control system enables the on/off functionality of any device element. Plasma Technology Development Company (Tehran, Iran) designed, developed, and manufactured the device.

### 2.2. Optical emission spectroscopy (OES)

Optical Emission Spectroscopy (OES) is a powerful, non-invasive diagnostic technique that analyzes the light emitted from electronically excited species in cold atmospheric plasmas to determine their composition and key parameters. In this method, the inherent glow of the plasma—resulting from electron impact excitation—is dispersed into its characteristic spectral lines using a spectrometer, allowing for the identification of various atoms, molecules, and radicals (e.g., OH, O, and N<sub>2</sub>) based on their unique emission wavelengths. By comparing the measured intensities with known reference spectra and employing calibration methods such as actinometry and OES enables researchers to identify the reactive species in the plasma environment (Kim et al., 2020). Thus, in the present study, the optical emission spectra in the 200–980 nm range of the DBD plasma device were recorded under ambient conditions using a single-channel spectrometer (model V550) with a resolution of 0.6 nm, manufactured by OPTC in Iran.

### 2.3. Materials

All chemical materials and solvents used in this study were obtained from Sigma-Aldrich (St. Louis, MO, US) and Merck (Darmstadt, Germany). These sources provided the required grades and quantities for all experimental procedures. All chemicals and solvents were of analytical grade and used without further purification.



**Fig. 1.** A) Schematic of the cold atmospheric pressure plasma decontamination system, B) Real-time snapshot of the system while processing the pistachios, C) Reactive species produced as a result of plasma generation.

## 2.4. Pistachio and sample preparation

Raw pistachio (*Pistacia vera* L.) of the Akbari type was purchased from a supplier in Kerman province, Iran, in the fall of 2023. Afterward, the pistachios were oven-dried at 30 °C for five days. To maintain a relative humidity (RH) of 65–70 % and a temperature of  $4 \pm 1$  °C, the pistachios were stored under controlled conditions and protected from sunlight until further experiments.

## 2.5. Plasma treatments

For each CP treatment, approximately 300 g of open-shell pistachios were uniformly distributed in a single layer on a glass tray, as illustrated in Fig. 1. The pistachios were then treated with a DBD plasma source (as described in Section 2.1) at a distance of 2 cm, 500 W power, 15 kV voltage, and 8 kHz frequency. Ambient air was utilized as the working gas. The samples were exposed to DBD plasma at various time intervals of 5, 10, 20, and 40 min.

All treatments in this study were performed in triplicate. An untreated open-shell pistachio sample served as the control group for the comparative analysis of nutritional and quality parameters. Sample preparation for biochemical testing and sensory evaluation was conducted in duplicate using the aforementioned methods to acquire a suitable quantity. The conditions and durations employed were based on the capabilities of the available equipment for microbial decontamination and aflatoxin elimination. These parameters significantly affect the efficiency of the plasma generation process (Diaz et al., 2022). To prepare the dried pistachio kernels for biochemical experiments, the tough pistachio shell was removed, and the kernels from each treatment were ground to a particle size of 2 mm using a grinder.

## 2.6. Biochemical and nutritional assay

$$\% N = [(mL H_2SO_4, \text{ sample} - mL H_2SO_4, \text{ blank}) \times \text{normality } H_2SO_4 \times 1.4007] / \text{weight sample (g)} \quad (1)$$

### 2.6.1. Fatty acid profile

The fatty acid profile was assayed using an AOAC gas chromatography method no. 996.06–1996 (Aoac 2002). In this method, the triacylglycerol (fat) and fatty acids were extracted from food, then methylated to fatty acid methyl ester using boron trifluoride in methanol, and FAMES were quantitatively measured by capillary gas chromatography (Huang et al., 2006). The sample was heated and stirred in a mixture of ethanol and 8 M HCl at 80 °C for 40 min, and then the mixture was allowed to cool. The sample was then transferred with ethanol into a Mojonnier fat extraction flask to facilitate liquid-liquid extraction using ethyl and petroleum ether. The resulting solution was then subjected to nitrogen evaporation. The extract was then saponified and methylated by introducing sodium hydroxide (0.5 M) in a methanol solution, followed by esterification with 14 % boron trifluoride in methanol. The fatty acids were converted into fatty acid methyl esters (FAMES) and quantified utilizing gas chromatography-mass spectrometry (GC–MS) (Nemzer and Al-Taher, 2023). The analysis of fatty acid methyl esters (FAMES) was performed using a Shimadzu gas chromatograph (GC-2010, Kyoto, Japan), which was coupled with a Shimadzu GCMS-QP2010 mass spectrometer and an Alltech ATe-Silar-90 capillary column (30 m, 0.25 mm diameter, 0.2 µm film thickness) (Alltech, Deerfield, IL). Electron ionization was conducted at an energy level of 70 eV. The injection was executed in split mode with a split ratio of 50, and the injection volume was set at 1 µL. Helium served as the carrier gas, maintaining a 0.6 mL/min column flow rate. To optimize the analytical parameters, three different temperature programs were employed:

namely, (1) isothermally at 180 °C; (2) 150 °C for 10 min, and then increased at 5 °C/min to 200 °C for 2 min; (3) 150 °C for 10 min, and then increased at 2.7 °C/min to 210 °C for 3 min. The optimal conditions were determined based on the resolution of trans fatty acids in the standard and subsequently applied to the analysis of the shortening sample (Huang et al., 2006). Analyses performed for quality control included examination of standard reference materials and blank samples, which were necessary to ensure accurate quantification of the FAMES. The quality index was calculated as the ratio of oleic to linoleic acid (O/L) (Esteki et al., 2018).

### 2.6.2. Total protein

Nitrogen contents in samples were measured by applying the micro-Kjeldahl method to determine the total amount of proteins according to AOAC Official Method 950.48. Briefly, the micro-Kjeldahl method was employed to quantify total protein content. To summarize, 0.1 g of the sample was placed into a micro-Kjeldahl flask. A catalyst composed of 0.42 g of CuSO<sub>4</sub> and 9.0 g of K<sub>2</sub>SO<sub>4</sub>, along with several glass beads to mitigate sample bumping, was added, followed by 15 mL of concentrated sulfuric acid (36 N).

The sample underwent digestion until the solution turned green (45–75 min), showing visibility of complete oxidation of the organic matter. After this, the digest was diluted with 50 mL of distilled water, and then the micro-Kjeldahl flask was connected to a distillation apparatus. Distillation was carried out by adding 45 mL of 15 N sodium hydroxide to allow the ammonia produced to be collected in a solution of boric acid containing methylene blue and methyl red indicators. Subsequently, the borate anion, corresponding to the nitrogen content, was titrated with a standardized 0.1 N sulfuric acid solution. For quality control, a reagent blank was also processed concurrently. The nitrogen content of the sample was determined using the formula (Venkatachalam and Sathe, 2006).

$$\% \text{ Protein} = \% N \times \text{appropriate factor (5.3)} \quad (2)$$

### 2.6.3. Total carbohydrate

The sample was hydrolyzed with 2 % sulfuric acid at 30 °C for 24 h. After hydrolysis, the liquid fraction of the hydrolysate samples was filtered and collected, and its sugar content was determined based on the Fehling method. The hydrolyzed filtered sample solution was 50 mL, of which 4 M NaOH and 2.5 M HCl were used to neutralize, and the volume diluted to 300 mL and then transferred to the burette. In an Erlenmeyer flask was mixed 5 mL of the Fehling A solution made from dissolving 34.6 g of copper (II) sulfate pentahydrate in 500 mL of distilled water, 5 mL of Fehling B solution made from dissolving 125 g of potassium hydroxide and 173 g of potassium sodium tartrate tetrahydrate in 500 mL of distilled water, and methylene blue indicator was added to this mixture. The burette solution was titrated in the flask in boiling conditions until a clear blue color was no longer seen. When a brick red color was observed, the volume was recorded. The following equation was utilized to calculate the sugar content of each sample based on the data obtained (Woldu and Tsigie, 2015). V is titration volume (mL), and f is Fehling factor (0.051).

$$\text{Sugar content (\%)} = (300 \text{ mL.f}) / V \times 100 \quad (3)$$

### 2.6.4. Chlorophylls and carotenoids

The amount of pigment was estimated following the method

described by Sumanta et al. (2014). At the first stage, 0.5 g of ground sample in 10 mL of 80 % v/v acetone were introduced into the evacuation system, after which stirred and centrifuged at 10,000 rpm for fifteen minutes. After that, 0.5 mL of the received supernatant was taken, and 4.5 mL of 80 % acetone was added to it; the absorbance of the received solution was determined at wavelengths of 663, 645, and 470 nm using a spectrophotometer. The quantity of pigments was then determined using the formula below: Chl-a, chlorophyll, and Chl-b, chlorophyll b.

$$\text{Chl a} = 12.25A_{663.2} - 2.79A_{645} \quad (4)$$

$$\text{Chl b} = 21.5A_{645} - 5.1A_{663.2} \quad (5)$$

$$\text{Total Chl} = \text{Chl a} + \text{Chl b} \quad (6)$$

$$\text{Total carotenoids} = (1000A_{470} - 1.82\text{Chl a} - 85.02\text{Chl b})/198 \quad (7)$$

#### 2.6.5. Malondialdehyde (MDA)

The manner of Heath and Packer (1968) was utilized to assay MDA (Heath and Packer, 1968). In this method, 0.5 g of sample was ground in 5 mL of trichloroacetic acid (20 %) containing thiobarbituric acid (0.5 %). The sample was centrifuged for 15 min at 6000 g. The supernatant was placed in hot water at 80 °C for 25 min. After immediate cooling with ice, it was centrifuged for 5 min at 6000 g. The absorbance of the sample was read at 600 and 532 nm. The final concentration of MDA was calculated using the following equation:

$$\text{MDA concentration}(\mu\text{mol per g of wet weight}) = ((A_{532} - A_{600}) / 1550) * 1000 \quad (8)$$

#### 2.6.6. Peroxide value

The peroxide value (PV) was determined iodometrically according to AOAC method (965.33) (Aoac 2012a). A 5 g sample was dissolved in a 2:1 (v/v) mixture of isooctane and glacial acetic acid. Potassium iodide was added to release iodine from peroxides. The liberated iodine was then titrated with a standardized sodium thiosulfate solution in the presence of a starch indicator. The endpoint was attained upon the disappearance of the blue color. The peroxide value was calculated using the formula

$$\text{PV} = ((V \times N) \times 1000)/W \quad (9)$$

V: volume of sodium thiosulfate (mL), N: normality of the sodium thiosulfate, and W: sample weight (g).

#### 2.6.7. Acidity value

The acidity value (AV) method gives an understanding of the quality and freshness of the oil. According to AOAC 940.28 (Aoac 2012b), a 5 g sample was dissolved in anhydrous diethyl ether and titrated with standardized KOH and phenolphthalein as indicators. The endpoint was reached when a persistent, faint pink color appeared. The acidity value was calculated using the formula:

$$\text{AV} = (V \times N \times 56.1)/W \quad (10)$$

V: volume of KOH (mL), N: normality of the KOH, 56.1: molecular weight of KOH, and W: sample weight (g).

#### 2.6.8. Humidity

The moisture content was determined according to AOAC method (925.40) (Aoac 1925). A 100 g sample was ground to a particle size of <3 mm. A tared porcelain dish was dried in an oven at  $103 \pm 2$  °C for 1 h, cooled in a desiccator, and weighed. The ground sample was added to the dish, weighed, and dried in the oven at  $103 \pm 2$  °C for 3 h. The dish was allowed to cool in a desiccator and then weighed. This process was done cyclically until the variation in weight between two successive weighings was within 0.005 g. The moisture content was determined

from the following formula:

$$\text{Moisture Content}(\%) = (\text{Initial Weight} - \text{Final Weight})/(\text{Initial Weight}) * 100 \quad (11)$$

#### 2.6.9. Sensory attributes

Sensory evaluation of pistachio smell and taste was conducted according to ASTM standards method. Twenty pistachio kernels were randomly selected in the assessment under standardized conditions (20–22 °C,  $40 \pm 5$  % relative humidity, consistent lighting) (Mohammadi Moghaddam et al., 2016; ASTM., 2021). To analyze the taste of the samples, a group of trained sensory evaluators evaluated the samples and used a standardized scale or descriptive words (Penci et al., 2013). Samples were randomized to reduce bias, and several evaluations were performed to guarantee reliability. Analysis was conducted to determine the average taste profile of the samples. Appearance involves color, size, shape, surface texture, and the evenness of color, all of which contribute to the visual appeal and perceived quality of the nuts evaluated in this study (Shakerardekani et al., 2013).

#### 2.7. Measurement of enzymatic antioxidant activity

##### 2.7.1. Ascorbate peroxidase assay (EC 1.11.1.11)

The enzyme assay was extracted from 200 mg of the sample, which was mixed with 50 mM potassium phosphate buffer (5 mL, pH 7.8) containing 1 mM ascorbic acid (maintaining optimal redox conditions), one mM phenylmethane sulfonyl fluoride, and 1 % polyvinylpyrrolidone (PVP, a clarifying agent). Afterward, the reaction mixture was centrifuged at  $22,000 \times g$  for 10 min at 4 °C. The collected supernatant was further dialyzed using cellophane membrane tubing for 240 min against cold extraction before the enzyme assay could be performed.

This reaction was initiated by adding 2 mM of hydrogen peroxide to the reaction medium, which consisted of the enzyme extract, 100 mM Tris-acetate buffer maintained at pH 7.0-, and 2-mM ascorbic acid. The change in extinction at 290 nm was recorded over 100 s employing the spectrophotometer. The extinction coefficient of  $2.8 \text{ (mM}\cdot\text{cm)}^{-1}$  was used to calculate the reaction. The specific enzyme activity was expressed as a unit per milligram (U /mg) of protein (Haida and Hakimian, 2019).

##### 2.7.2. Catalase assay (EC 1.11.1.6)

The catalase assay was performed by homogenizing fresh samples (200 mg) in 5 mL of 50 mM Tris-NaOH buffer (pH 8.0) that included 0.5 % (v/v) Triton X-100 (a detergent), 2 % (w/v) PVP, and 0.5 mM EDTA (a chelating agent). The homogenate was centrifuged for 10 min at 4 °C at  $22,000 \times g$ ; the supernatant was then subjected to dialysis before enzyme activity was assayed.

The enzyme activity was assessed using a reaction mixture of 1 mL containing 50 mM of potassium phosphate buffer (pH 7.0), and 250  $\mu\text{L}$  of enzyme extract was initiated by introducing 60 mM of hydrogen peroxide. The optical density was then measured using a UV spectrophotometer at 240 nm for 3 min. The decomposition of  $\text{H}_2\text{O}_2$  was calculated using an extinction coefficient of  $39.4 \text{ (mM}\cdot\text{cm)}^{-1}$ . One unit of activity corresponds to 1 mM of  $\text{H}_2\text{O}_2$  decomposed per minute and was represented as U /mg of protein (Haida and Hakimian, 2019).

#### 2.8. Statistical analyses

Initially, the normality of the data was evaluated using the Kolmogorov-Smirnov and Shapiro-Wilk tests. Following confirmation of normality, the results were evaluated using analysis of variance (ANOVA). Statistical analyses were performed on three replicates of data obtained from each treatment (control, 5-, 10-, 20- and 40-minute plasma treatment) using SPSS Statistics software (v. 27). Comparisons



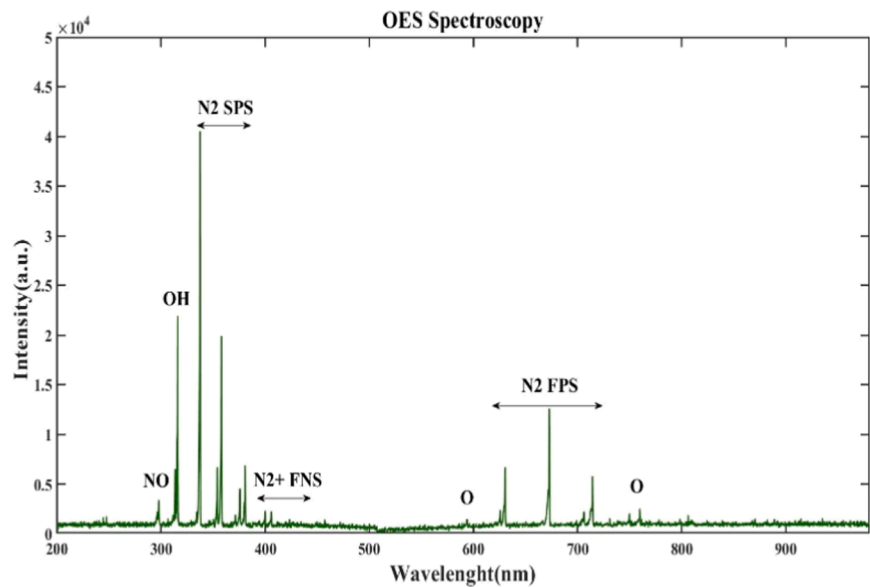


Fig. 2. Optical emission spectrum of the DBD plasma device.

among groups were made using a one-way ANOVA followed by Duncan’s post hoc test. P-values of \* < 0.05 and \*\* < 0.01 were considered statistically significant.

3. Results and discussion

3.1. Optical emission spectra

Fig. 2 shows the OES optical emission spectrum from the plasma generated in the DBD device. For more precise species identification in the plasma environment, spectral measurements were performed in the 200–980 nm range to display the intensity of the electromagnetic radiation produced by the excited species in the plasma across the UV, VIS, and NIR regions. This is because the radiation emitted during the relaxation of some excited species to their ground state (such as N<sub>2</sub>-SPS, N<sub>2</sub>-FNS, and the OH radical) falls within the ultraviolet wavelength range, while others (like O and N<sub>2</sub>-FPS) appear in longer wavelength regions. NOx species are dominant in DBD plasma. The spectrum displays peaks related to N<sub>2</sub> and N<sub>2</sub><sup>+</sup>, mainly caused by high levels of molecular nitrogen in the air. The peak formed at a wavelength of 283 nm is related to nitrogen oxide (NO) (Liu et al., 2020), and the peak formed at 309 nm corresponds to hydroxyl radicals (OH) (Zigon et al., 2019). OH, radicals are generally produced through the interaction of plasma and moisture in the air. The peaks formed in the range of 337–380 nm are due to the transitions of the second positive system (SPS) of nitrogen between the C<sup>3</sup>Π<sub>u</sub>→B<sup>3</sup>Π<sub>u</sub> levels and the relatively more minor peaks

formed in the range of 391–470 nm are related to the transitions of the first negative system (FNS) of nitrogen between the B<sup>2</sup>Σ<sub>u</sub><sup>+</sup>→X<sup>2</sup>Σ<sub>u</sub><sup>+</sup> levels. The visible peaks around 590 nm and the 750–800 nm range are related to oxygen species (excited oxygen and singlet oxygen) (Cvelbar et al., 2006). The remaining peaks in the spectrum are derived from nitrogen transitions, which is expected considering air as the plasma gas in the device (Lamichhane et al., 2024). Furthermore, the more apparent peaks formed in the range of 625–715 nm correspond to the transitions of the first positive system (FPS) of nitrogen between the B<sup>3</sup>Π<sub>u</sub>→A<sup>3</sup>Π<sub>u</sub> levels. The spectrum is dominated by the peaks related to the N<sub>2</sub> Second Positive System (SPS) and N<sub>2</sub><sup>+</sup> First Negative System, primarily due to the very high concentration of molecular nitrogen in the atmosphere.

3.2. Fatty acids change after DBD plasma treatment

Oil is one of the main derived products in nuts, and therefore, the quality and characteristics of its fatty acid profile are essential. On the other hand, sterilization can alter some desirable attributes of oilseeds, especially their fatty acid composition (Rioux and Legrand, 2007). CP technology can influence the composition and stability of saturated fatty acids in food products.

The fatty acids, both saturated and unsaturated, derived from the analysis of pistachios are presented in Table 1. The pistachio samples studied contain eight compounds of saturated fatty acids, including palmitic acid (C16:0), stearic acid (C18:0), arachidic acid (C20:0), and myristic acid (C14:0), and unsaturated fatty acids, including linoleic

Table 1  
Duncan’s tests of the effect of DBD on fatty acid profile.

Plasma treatment (minute)					
Pistachio fatty acid profile	0	5	10	20	40
Myristic acid ( % )	0.10 <sup>a</sup> ±0.000	0.11 <sup>a</sup> ±0.001	0.11 <sup>a</sup> ±0.006	0.10 <sup>a</sup> ±0.006	0.1 <sup>a</sup> ±0.000
Palmitic acid ( % )	10.41 <sup>b</sup> ±0.22	10.19 <sup>b</sup> ±0.73	10.32 <sup>b</sup> ±0.03	10.31 <sup>b</sup> ±0.01	11.25 <sup>a</sup> ±0.39
Palmitoleic acid ( % )	0.85 <sup>a</sup> ±0.03	0.86 <sup>a</sup> ±0.04	0.93 <sup>a</sup> ±0.07	0.86 <sup>a</sup> ±0.02	0.86 <sup>a</sup> ±0.05
Stearic acid ( % )	1.24 <sup>a</sup> ±0.02	1.19 <sup>a</sup> ±0.03	1.23 <sup>a</sup> ±0.01	1.24 <sup>a</sup> ±0.01	1.26 <sup>a</sup> ±0.02
Oleic acid ( % )	51.40 <sup>a</sup> ±0.16	51.19 <sup>a</sup> ±0.67	51.17 <sup>a</sup> ±0.72	50.87 <sup>a</sup> ±0.38	52.25 <sup>a</sup> ±0.05
Linoleic acid ( % )	35.29 <sup>a</sup> ±0.40	35.35 <sup>a</sup> ±0.61	34.79 <sup>a</sup> ±0.49	35.43 <sup>a</sup> ±0.39	33.48 <sup>b</sup> ±0.14
α-Linolenic acid ( % )	0.78 <sup>a</sup> ±0.15	0.83 <sup>a</sup> ±0.01	0.84 <sup>a</sup> ±0.01	0.86 <sup>a</sup> ±0.02	0.82 <sup>a</sup> ±0.01
Arachidic acid ( % )	0.30 <sup>a</sup> ±0.08	0.34 <sup>a</sup> ±0.01	0.32 <sup>a</sup> ±0.02	0.36 <sup>a</sup> ±0.03	0.25 <sup>a</sup> ±0.01
P index	1.46 <sup>b</sup> ±0.02	1.45 <sup>b</sup> ±0.04	1.47 <sup>b</sup> ±0.04	1.44 <sup>b</sup> ±0.03	1.56 <sup>a</sup> ±0.01

\* Means followed by the same letter are not significantly different (p < 0.01).  
\* Data represent mean ± SD (n = 3).

acid (C18:2),  $\alpha$ -linolenic acid (C18:3), oleic acid (C18:1), and palmitoleic acid (C16:1). Indirect DBD treatment did not lead to any notable changes in the levels of palmitoleic acid,  $\alpha$ -linolenic acid, arachidic acid, myristic acid, oleic acid, and stearic acid across all treatment groups compared to the control group.

No significant differences were detected in the palmitic and linoleic acid levels when comparing the 5- to 20-minute treatments with the control group. However, following a 40-minute treatment, palmitic acid levels increased to 11.25 %, while linoleic acid levels decreased to 33.48 %, compared to 10.41 % and 35.29 % in the control sample. Thus, it can be concluded that fatty acids remained largely unaffected during short treatment times (5–20 min). In contrast, only two of the eight fatty acids changed after 40 min of treatment, with increases and decreases of approximately 8 % and 5 % for palmitic acid and linoleic acid, respectively.

Saturated fatty acids have carbon chains filled with hydrogen atoms and contain no double bonds between them (Rioux and Legrand, 2007). Unsaturated fatty acids contain one or more double bonds in their carbon chains, significantly affecting their chemical and biological properties. Based on the number of double bonds, there are two types of unsaturated fats: monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA), the first containing one double bond and the other containing more than one double bond. This structural difference is crucial in their reactivity and interactions with different molecular structures (Wang et al., 2024).

CP, through the ionization of gas molecules, generates free radicals. The radicals are highly reactive, can initiate chemical reactions with lipids, and may cause oxidative stress (Portal et al., 2022). The oxidation of unsaturated fatty acids can be divided into several categories. Primarily, lipid oxidation is an autocatalytic free-radical process characterized by three stages: initiation, propagation, and termination (O'Brien and O'Connor, 2022). During the initiation stage, species such as hydroxyl radicals ( $\bullet\text{OH}$ ) attack the double bonds of the fatty acids and form a substance called hydroperoxides (Nah et al., 2013). These lipid radicals further oxidize molecular oxygen ( $\text{O}_2$ ) into lipid peroxy radicals, which in turn remove hydrogen from other lipids, thus initiating a chain reaction. In the propagation phase, these hydroperoxides decompose into several volatile compounds, resulting in rancidity (Assi et al., 2023). Finally, stabilization occurs in the termination stage by combining two radicals to form a non-radical product and forming secondary oxidation products of aldehydes and ketones. The contribution of peroxide and peroxy radicals, and alkoxy radicals is a complex reaction defining lipid oxidation. The alkoxy radical mechanism is fundamental in the propagation and termination phases (Wang et al., 2024).

Our investigation shows that the mechanism described during the 40 min of plasma treatment specifically influenced linoleic acid, a polyunsaturated fatty acid characterized by a higher number of double bonds, resulting in a statistically significant reduction in its concentration. RONS, particularly singlet oxygen and hydroxyl radicals, attack the C—H bond in the methyl group and remove the hydrogen atoms. Because it also requires much less energy to remove a hydrogen atom from C—H bonds that lie between the double bonds, the more double bonds a fatty acid has (such as  $\alpha$ -linolenic acid and linoleic acid), the more susceptible they are to RONS (Surowsky et al., 2016). Studies indicate that, with the use of CP, the levels of lipid oxidation go up alongside the level of saturated fatty acids. In contrast, the level of unsaturated fatty acids, which are known to be sensitive to oxidation, decreases (Sang et al., 2024). It is established that the oxidation of unsaturated fatty acids contributes to increased saturated fatty acids through different pathways. This typically occurs through the reaction of molecular oxygen with the double bonds of the unsaturated fats, forming very unstable hydroperoxide. As observed in this study, these hydroperoxides can further degrade into saturated fatty acids [36] as an increase in palmitic acid as a saturated fatty acid. In this regard, increases in linoleic, stearic, palmitic, and oleic acid content were detected

in cashew nuts during low-pressure plasma treatment using glow discharge plasma (Alves Filho et al., 2019). Gebremical et al. (2019) noted that peanut fatty acids reduced in unsaturated fatty acids and slightly increased in saturated fatty acids due to multihollow surface dielectric barrier discharge.

The ratio of oleic to linoleic acid (O/L), called the “quality index,” assayed in this work, is reported in Table 1. This experiment demonstrated that extending the duration of plasma treatment to 40 min resulted in a statistically significant enhancement of this parameter (6.84 % increase compared to control).

The O/L ratio is an important quality index in assessing the nutritional and technological quality of a range of oils, especially pistachios and peanut oils. The pistachio quality is related to the amounts of oleic acid (O) and linoleic acid (L), as these make up the most significant percentage of fatty acids in this nut (Roosban et al., 2005). The oxidative rancidity of most nut oils increases with increasing levels of polyunsaturated fatty acids. In groundnuts, a strong negative relationship was observed between oleic and linoleic acid, suggesting that increasing oleic acid content leads to a decrease in linoleic acid (Gangadhara and HL, 2018). Several studies show that higher O/L ratios positively affect oil quality and stability (Esteki et al., 2018). On the other hand, the oleic to linoleic acid ratio can estimate the storage period and stability of the oil. In this investigation, extending the plasma treatment duration to 40 min enhanced this parameter, thereby prolonging the shelf life of pistachios. In the practical implementation of CP technology in food processing, it is crucial to identify the key trade-offs between the increased product shelf life and the improved nutritional content characteristic of foods.

### 3.3. Total protein change after DBD plasma treatment

The variations in protein content resulting from different durations of plasma treatment are presented in Table 2. Five- and ten-minute plasma treatments did not result in statistically significant differences in protein content compared to the protein content compared to the control. In contrast, treatment durations of 20 and 40 min resulted in a statistically significant reduction. The observed reductions in protein content compared to the control were 12.29 % for the 20-minute treatment and 14.56 % for the 40-minute treatment.

Protein structure and function change due to the involvement of CP by one or more processes. Oxidative modifications, structural changes, and the activation of molecular chaperones are among these mechanisms. In oxidative modifications, CP creates ROS that interact with amino acids, subsequently undergo oxidation, and form structural alterations (Guo et al., 2023). Methionine and cysteine, two amino acids containing sulfur, can be oxidized to sulfonic acids, and the aromatic amino acid tyrosine can undergo H-abstraction (Guo et al., 2023).

Acharjee et al. (2023) showed that non-thermal pin-to-plate CP treatment increased solubility and emulsifying activity, as evidenced by a 66.94 % increase in soluble protein in pea protein isolate. The study referenced shows protein has structural rearrangements. The FTIR spectrum indicates that CP alters protein conformation, particularly in the amide I region. CP increases the activity of the molecular chaperones, including Hsp33, that are known to have pivotal roles in mitigating protein aggregation. Nevertheless, exposure to CP may extend longer and cause non-specific oxidation, which inactivates these proteins (Dirks et al., 2023).

In our research, the observed reduction in protein levels following 20 and 40 min of DBD treatment appears to be associated with the initial mechanism, wherein oxidative modifications and structural changes contribute to this decline. A majority of research in this area acknowledges that CP treatment improves the functional properties of proteins and nutritional integrity (Bu et al., 2023; Ji et al., 2018; Sharafodin and Soltanizadeh, 2022). However, the question regarding the desirability of the observed protein content reduction after both 20 and 40 min of DBD treatment is still open and warrants further investigation. In this context,

**Table 2**

Duncan's tests of the effect of DBD on nutritional assessment of pistachio.

Plasma treatment (minute)					
Nutritional assessment of pistachio	0	5	10	20	40
Total protein ( %)	24.4 <sup>a</sup> ±1.17	24.72 <sup>a</sup> ±0.972	24.60 <sup>a</sup> ±0.23	21.40 <sup>b</sup> ±0.92	20.85 <sup>b</sup> ±0.41
Total carbohydrate ( %)	4.36 <sup>a</sup> ±0.80	3.93 <sup>ab</sup> ±0.55	3.52 <sup>ab</sup> ±0.27	3.40 <sup>b</sup> ±0.20	2.44 <sup>c</sup> ±0.15
Chlorophyll (mg/mL)	30.17 <sup>a</sup> ±1.23	20.66 <sup>a</sup> ±5.16	27.06 <sup>a</sup> ±3.06	29.93 <sup>a</sup> ±3.17	28.05 <sup>a</sup> ±6.76
Carotenoid (mg/mL)	3.44 <sup>a</sup> ±0.21	3.15 <sup>a</sup> ±0.76	2.84 <sup>a</sup> ±0.43	3.21 <sup>a</sup> ±0.28	3.16 <sup>a</sup> ±0.95
Malondialdehyde (μmol/g)	0.97 <sup>a</sup> ±0.16	0.82 <sup>a</sup> ±0.07	0.92 <sup>a</sup> ±0.07	1.03 <sup>a</sup> ±0.06	1.05 <sup>a</sup> ±0.08
Acidity value ( %)	0.36 <sup>a</sup> ±0.12	0.34 <sup>a</sup> ±0.04	0.33 <sup>a</sup> ±0.06	0.39 <sup>a</sup> ±0.12	0.36 <sup>a</sup> ±0.06
Moisture ( %)	4.31 <sup>a</sup> ±0.13	4.31 <sup>a</sup> ±0.10	4.33 <sup>a</sup> ±0.14	4.24 <sup>a</sup> ±0.07	4.28 <sup>a</sup> ±0.06

\* Means followed by the same letter are not significantly different ( $p < 0.01$ ).\* Data represent mean  $\pm$  SD ( $n = 3$ ).

the protein content remained stable during treatment periods of up to 10 min, even in the presence of RONS and RNS. This implies producing reactive species was not enough to negatively impact protein levels. Studies also have revealed that some amino acids, such as methionine and, to some extent, tryptophan, might act as endogenous antioxidants after oxidation. They can convert their electrons to neutralize the reactive oxygen species ROS, which protects cells and tissues from breakdown (Guidea et al., 2020; Surowsky et al., 2016).

### 3.4. Total carbohydrate change after DBD plasma treatment

The assessment of carbohydrate content conducted on pistachios shows a clear trend of decreasing carbohydrate levels with increasing duration of DBD treatment (Table 2). However, this decline was statistically significant only after 20 and 40 min of DBD treatment compared to the control. Notably, compared to the control, the 40-minute treatment resulted in a greater reduction in carbohydrate levels (up to 44.0 %) compared to the 20-minute treatment (22.03 %).

The total carbohydrate content in pistachios varies across different studies. The carbohydrate content comprises a relatively high content of sucrose, glucose, and galactose, of which sucrose is present in all tissues of the pistachio tree in the most significant quantities (Guney et al., 2023). General mechanisms of CP interaction with organic molecules include carbohydrate degradation, structural modifications, and cross-linking. The reactive species generated by DBD can interact with the chemical bonds in carbohydrates and, therefore, cause devastation of carbohydrates with a consequent decrease in their total amounts (Surowsky et al., 2016). CP is also capable of causing chemical modifications of carbohydrates. For instance, it could change some of the glycosidic bonds or change the functional groups of the carbohydrate molecules. CP treatment has the potential to cause peripheral activation and the formation of covalent bonds between the C—C starch chains or between carbohydrates and other components of the food matrix (Oner et al., 2023). In our research, the observed reduction in total carbohydrates during DBD treatment, particularly at the 20- and 40-minute, appears to be associated with the initial mechanism (carbohydrate degradation). The present discussion, therefore, holds importance in understanding the impacts of CP on carbohydrates in various fields. It can enhance food conservation technology and promote recognition in agriculture and pharmaceuticals.

### 3.5. Pigments (Chlorophylls and carotenoids) after DBD plasma treatment

Table 2 indicates that the application of DBD plasma treatment to pistachios did not induce substantial alterations in the levels of chlorophyll or carotenoids. These pigments, therefore, remained relatively stable despite the DBD plasma process.

Compared to other nuts, pistachios contain a high concentration of two important pigments and antioxidants: chlorophylls and carotenoids. Carotenoids, which mainly contain compounds in lutein and zeaxanthin, are related to antioxidant and health effects (Fallico et al., 2011). CP

treatment can alter the chlorophyll content of nuts like pistachios and, following that, the colors and stability of the nuts. It can affect the visual appeal of the nuts and even their nutritional or caloric value, respectively (Foligni et al., 2022). Observing the reduction of photosynthetic pigments after plasma treatment is common in various studies (Makari et al., 2021; Ramazzina et al., 2015), the reason for which may be attributed to the pigment oxidation and degradation by RONS (Foligni et al., 2022). However, in our evaluation, no change in the pigments was observed due to the utilization of indirect plasma. It should be noted that the penetration depth of CP is limited, and the main effect of plasma on food is superficial. Therefore, it can remarkably remove surface contaminants without damaging food products (Foligni et al., 2022).

### 3.6. Malondialdehyde after DBD plasma treatment

The experiment revealed a rise in MDA levels corresponding to extended DBD plasma treatment. While MDA showed an upward trend, the recorded changes lacked statistical significance, as presented in Table 2. Therefore, although a pattern was observed, the increase in MDA was not considered a significant result of the DBD plasma treatment.

MDA is one of the aldehyde products that causes cellular damage through proteins, lipids, and DNA biomolecules. This makes it a useful marker and one of the best predictors of oxidative stress and cellular damage in different biological systems by ROS (Chen et al., 2015).

According to studies, no threshold limit of MDA produces optimum damage in pistachio, but a greater quantity of MDA is proportional to the raised oxidative stress and lipid peroxidation. CP treatment has been found to increase the MDA content in pistachios. The elevation of this factor indicates that the reactive species generated during plasma treatment can induce lipid peroxidation and consequently lead to raised MDA concentration. Thus, the treatment conditions, including the time and plasma power, could affect the degree of lipid peroxidation followed by MDA content (Foligni et al., 2022; Makari et al., 2021).

In the present investigation, it appeared that oxidative stress remained unchanged throughout all CP treatment durations, thereby further supporting the conclusion that CP does not influence lipid oxidation. CP treatment significantly impacted the reduction of linoleic acid within a 40-minute timeframe ( $p < 0.05$ ). Evidence indicates that this decrease was accompanied by the immediate transformation of linoleic acid to palmitic acid and the production of a small quantity of MDA that has a toxic effect on cellular organelles. In addition, pistachios are rich in antioxidants, such as tocopherols, flavonoids, and resveratrol. These compounds inhibit lipid oxidation and protect against oxidative spoilage (Noguera-Artiaga et al., 2019).

Therefore, during our investigation treatment time, pistachios detoxified and sterilized with DBD can be packed with essential nutrients, including vitamins, minerals, and healthy fats. While minimal MDA production can maintain their nutrients, making pistachios maintain their status as healthy food (Nadimi et al., 2019).

### 3.7. Peroxide and acidity value after DBD plasma treatment

The peroxide and acidity values of pistachios are important determinants of quality and freshness, as well as food safety (Sharma and Jain, 2015). The peroxide value is an index of the oxidative status of the oil in the pistachios. As the amount of oxidation increases, the peroxide value also increases, and this makes the nuts become rancid and of poor quality (Tavakolipour et al., 2017). This study measured the peroxide number as zero in all tested samples (both control and treatment).

According to some studies, CP treatments cause a slight increase in the peroxide values, but these changes are not statistically different, and the obtained values are still within acceptable limits (Bora et al., 2022). In our investigation, the peroxide value is the minimum degree (zero), which indicates the freshness and very high quality of the samples and the absence of any fat oxidation. Hydroperoxides were found to be slightly elevated in pistachio kernels in the study by Foligni et al., 2022; however, it appears that oxidation is not a severe problem under controlled conditions. Niveditha et al. (2023) studied the impact of CP treatment and mentioned there was a slight effect of CP treatment as there was only a minor upsurge in the peroxide value of palm oil after treatment. In other similar studies, several divergent results have been reported. For instance, exposure to multi-hollow surface dielectric barrier discharge plasma resulted in higher peroxide value in peanuts due to changes in fatty acid composition (Gebremical et al., 2019).

The acidity value, measured as the free fatty acid content value, indicates the degree of hydrolysis of the triglycerides in the sample and the level of hydrolytic rancidity in the oil of pistachios (Sharma and Jain, 2015; Yahyavi et al., 2020). A higher acidity value or acidity value means more hydrolytic rancidity and, hence, low quality.

The acidity value generally remained stable across various studies. For instance, no significant changes were observed in acidity value after CP treatment in palm oil (Niveditha et al., 2023). Similar findings were reported in other food products, where CP did not significantly alter the acidity value (Tappi et al., 2023). Our study revealed that CP processing did not yield a statistically significant effect on the acidity value compared to the control (Table 2). Therefore, CP processing can be considered a non-destructive technique with respect to acidity value.

### 3.8. Humidity and sensory attributes after DBD plasma treatment

Regarding humidity, DBD did not significantly reduce the humidity of pistachios over the 5 to 40-minute period. The moisture content for all treatments was within the recommended range (4–6 %) (Table 1). Additionally, no appreciable alterations in the sensory attributes (odor, taste, and appearance) of pistachios were distinguished. The water content of pistachios has a vital role in the quality of pistachios in terms of stability, color, flavor, texture, and weight (Gebremical et al., 2019). Plasma treatment may reduce pistachio moisture content due to mild heating and surface etching. The safe moisture content for pistachios, which have to be stored, usually ranges from 4–6 %. This range hinders the growth of microorganisms and preserves the quality of the nuts. (Tavakolipour, 2015). However, very low moisture content also causes an increased rate of rancidity reactions by oxidation and alters the flavor of the product (Gebremical et al., 2019). In this study, humidity fluctuation was relatively stable, ranging within the optimal setting for storage conditions (below 5 %). Gebremical et al. (2019) also mentioned that the moisture content in peanuts declined with the increase in power and duration of treatment but still remained near the optimal level of peanut storage.

Taste, odor, and appearance are basic factors that influence the acceptability and perceived satisfaction level of food products (Zhang et al., 2022). CP treatment can alter the sensory characteristics of nuts. These effects are attributed to variations in the chemical composition and structure of the nuts following exposure to the species generated during the plasma process. Changes occur due to the etching of biomacromolecules, cross-linking of biomacromolecules, oxidation of lipids

and proteins, and UV emission (Ling et al., 2016). Our finding indicated that the organoleptic characteristics of pistachio kernels remained unchanged for up to 40 min of plasma treatment, as the level of oxidation was insufficient to influence this parameter.

This result is supported by the findings of Foligni et al. (2022), where CP treatment was used in the treatment of nuts and found no changes in the qualities of nuts; thus, the treated nuts and untreated nuts had no significant differences. Research shows that CP does not cause substantial alteration to the appearance of nuts, a factor essential in marketing (Zhang et al., 2022). However, certain researchers claimed that the degrees of the effects depend on various treatment parameters. Further study of treatment protocols concerning different nut types could be beneficial.

### 3.9. Effects of DBD plasma on antioxidant enzyme activity

Enzymes are substances that are involved in numerous activities and promote biochemical processes. In this respect, antioxidant enzymes are the essential enzymes that maintain an adequate antioxidant defense in pistachio fruits to control cell redox processes.

#### 3.9.1. Ascorbate peroxidase

APX is capable of reducing hydrogen peroxide using ascorbate as the source of electron donors. It contributes to cellular protection against oxidative damage, in particular stress situations (Li, 2023). CP treatment has been shown to reduce the activity of enzymes, including antioxidant enzymes, in food systems. This decline is due to reactive species generated during the plasma treatment, which affects the conformation and functionality of the enzyme (Surowsky et al., 2013). These interactions are more apparent after prolonged exposure, resulting in a decline in enzyme activity (Farooq et al., 2023).

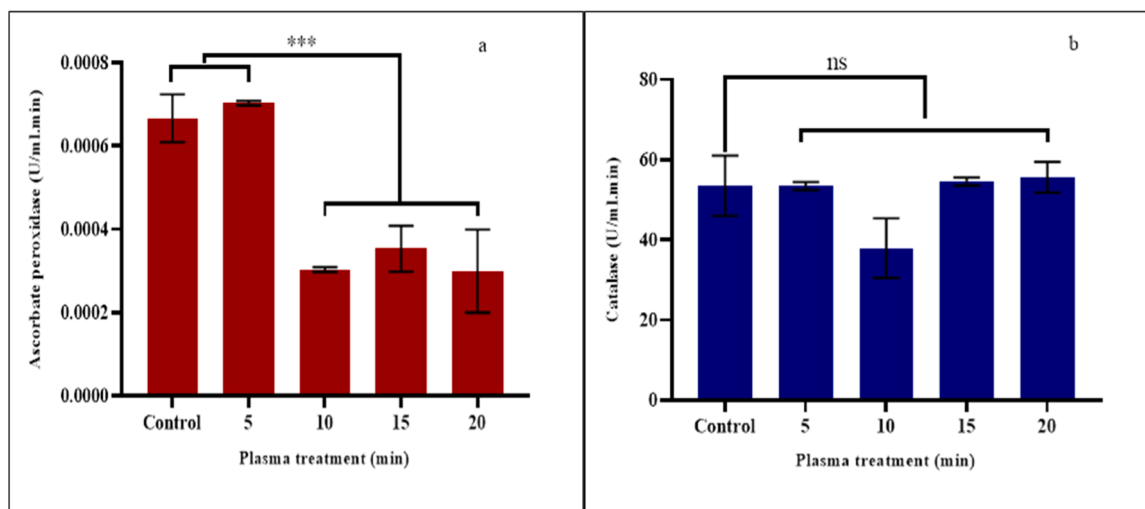
The data presented in Fig. 3a indicate that extending the DBD treatment to 5 min did not influence the activity of the ascorbate peroxidase enzyme. However, a statistically significant reduction was observed when the treatment duration was increased to 10 min compared to the control group (55 %). Ascorbate peroxidase also underwent reduction after 20 and 40 min of exposure to DBD treatment compared to the control. However, extending the treatment time from 10 to 40 min did not result in any statistically significant alterations. In this regard, in a model food system, the reduced activity of the enzyme by 90 % was observed after 180 s of CP treatment (Surowsky et al., 2013).

In summary, ROS influences ascorbate peroxidase function through several pathways: oxidative modification, protein degradation, conformational alteration, and regulation (Xiao et al., 2022). In oxidative modifications, cysteine residues in APX can be S-sulfonated, S-nitrosylated, or form a disulfide bond, leading to the conformation and function of the enzyme (Maruta and Ishikawa, 2017). High ROS produces protein oxidation and degradative mechanisms that affect the stability and functionality of APX (Maruta and Ishikawa, 2017). ROS also alters the protein-folded structure of APX. These changes could obstruct the active site of the enzyme and get in the way of the enzyme's relation with the substrate (Ansari et al., 2024). Furthermore, ROS can modulate specific APX isoforms via their conformational changes (regulatory mechanisms) in post-translational modifications such as phosphorylation, acetylation, and methylation or by directly or indirectly regulating enzymes that perform these modifications, ultimately affecting APX activity (Corpas et al., 2024).

This change in APX through DBD may not always be undesirable in the food industry. Unfortunately, there are no specific studies on the effect of CP treatment on APX in dried nuts; however, general observations in various food systems show that CP treatment decreases the activity of peroxidase enzymes, including APX (Kaur et al., 2021).

These reductions can be helpful in particular food processing uses where enzyme reactivity has to be controlled to boost preservation time or product quality.





**Fig. 3.** Effect of DBD on the activity of antioxidant enzymes. a) Ascorbate peroxidase, b) Catalase. Data represent mean  $\pm$  SD ( $n = 3$ ). \*\*\* $p < 0.001$  (Duncan's test), ns: non-significant.

### 3.9.2. Catalase

The variations in catalase activity during plasma treatment time are illustrated in Fig. 3b. The presence of plasma did not influence the activity of the catalase enzyme when compared to the control group. A minor reduction in catalase activity was recorded after 10 min of plasma treatment; however, this reduction did not reach statistical significance compared to the control group.

Catalase is an enzyme that works in the catalytic breakdown of hydrogen peroxide ( $H_2O_2$ ) into water ( $H_2O$ ) and oxygen ( $O_2$ ) (Baker et al., 2023). Stable structure and selective intracellular positioning of catalase efficiently degrade the reactive species and prevent oxidative stress in cells. Catalase is mainly found in peroxisomes, the job of which is to neutralize ROS. This way of packaging protects the enzyme from other structures of the cell and effectively preserves its function in combating ROS (Sharma and Ahmad, 2014). Second, the heme group of the catalase and its form as a tetramer afford the enzyme a good measure of protection against oxidative stress. The aforementioned structure facilitates the optimization of the activity of catalase and offers an efficient defense against increased levels of ROS (Scibior and Czacot, 2006). Finally, because of the high turnover rate of catalase, the enzyme can neutralize ROS before the reactive molecules can degrade cellular contents (Mahomoodally and MA-L, 2022). During the plasma treatment, catalase activity did not change compared to the control group due to the mechanisms described.

The stability of catalase in CP exposure depends on the food matrix and treating parameters, including plasma density, treating time, and other reactive particles within the plasma. In the condition of our investigation, shorter treatment times (below 40 min) may not significantly affect the enzyme's activity. Therefore, the extent of reactive species interaction and the resulting impact on enzyme activity can be limited if the enzyme's active site and overall structure remain largely intact (Han et al., 2019).

Although CP is mainly used to deactivate unwanted enzymes, it also shows the ability to activate enzymes in specific scenarios, the research of which is still to be undertaken. Such complexity implies the necessity of developing specific applications in food processing for the best results to be realized.

## 4. Conclusion

Cold plasma technology offers a promising approach to decontaminate microbes. However, this method has given some trade-offs that need to be considered carefully. The results of the present study

indicated that 5 and 10 min of DBD plasma treatment had no adverse effects on the assessed nutritional parameters, which means that these durations are safe in terms of decontamination. Extending treatment to 20 min may also be acceptable, provided that the modification of protein and carbohydrates can be ignored. However, ascorbic acid activity declined from 10 min onwards, but the fatty acid composition remained stable at 10 and 20 min. Moreover, no significant difference was noted in malondialdehyde concentrations, peroxide values, acidity, or sensory attributes following plasma treatments. Since pistachios do not play a major role as a source of vitamin C in an average diet, loss of some of the ascorbic acid can be viewed as a reasonable compromise toward microbial safety. Notably, 40 min of plasma treatment improved the P-index, indicating potential shelf-life extension. However, this must be weighed against possible nutritional degradation.

Several limitations of the current study should be noted. First, due to time limitations, the investigation of the long-term effects of the cold plasma on protein, carbohydrate, and fatty acid profiles during prolonged storage could not be conducted. Moreover, although prolonged exposure (40-minute duration) to DBD plasma could lead to some quality degradation, the alternative benefit of shelf-life extension is worthy of future study. Additionally, the expected influence of plasma on various antioxidants should also be investigated. Future research should focus on providing more comprehensive results by examining a variety of nuts and different plasma conditions, such as gas pressure, plasma power, and the proximity of the sample to the plasma source.

By offering valuable insights into the effects of cold plasma on pistachio quality, this research marks a significant advancement toward the development of safe and efficient uses of this technology in the food industry. The results of this examination will assist scholars in generating new knowledge and establishing industrial standards in this field.

## Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

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## Ethical statement

The research presented does not involve any animal or human study.

## CRediT authorship contribution statement

**Mahdiyeh Bakhtiyari-Ramezani:** Writing – review & editing, Supervision, Resources, Project administration, Investigation, Funding acquisition, Conceptualization. **Fatemeh Amani:** Writing – review & editing, Methodology, Investigation, Formal analysis, Data curation. **Aboutorab Naeimabadi:** Writing – review & editing, Methodology, Investigation.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have influence the work reported in this paper.

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## Data availability

Data will be made available on request.

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