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Vacuum Plasma Investigation for Pistachio Microorganisms Inactivation: Physical and Chemical Study

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ABSTRACT

This study aimed to evaluate the effect of Vacuum Non-thermal Plasma (VNP) on surface decontamination of pistachios. Food safety is considered as a critical issue in food industry, and detoxification is an essential step during food processing. VNP is an effective method on decontamination of food and food processing. In this experiment, the ability of VNP to inactivate highly resistant indicator bacteria *Geobacillus stearothermophilus* and pistachio contaminating microorganisms was investigated. Firstly, the effect of Vacuum Non-thermal Air Plasma (VNAP) on G. stearothermophilus was investigated at power of 30, 50, 70, and 90W for 10, 20, 30, 40, and 50 minutes. The results indicate that the minimum dose of VNAP to completely inactivate G. *stearothermophilus* was 30 minutes and 50W. So, it can be considered as an Optimum Dose (OD) for VNAP treatment. Thereafter, OD was used to investigate the effects of VNAP and then Nacuum Non-thermal Oxygen Plasma (VNOP) on pistachio contaminating microorganisms. The pistachios samples exposed to VNAP in OD (50W for 30 minutes) showed 1.0 log microbial reduction, while the samples exposed to VNOP in OD showed 2.0 log reduction. Both VNAP and VNOP applied significantly decreased microbial load of pistachios, however, VNOP was more effective than VNAP. Therefore, VNOP in 50W for 30 minutes can be chosen as an OD. Pistachio samples were also analyzed by physicochemical properties in OD. In conclusion, VNP was found to be effective on decontamination of pistachios, with no significant effects on its properties.

Keywords: *Geobacillus stearothermophilus*, Optimum Dose (OD), Vacuum Non-thermal Air Plasma (VNAP), Vacuum Non-thermal Plasma (VNP), Vacuum Non-thermal Oxygen Plasma (VNOP).

INTRODUCTION

Pistachios are known as an important source of energy, nutrients, and antioxidants that are essential for human health (Baynes, 1991). Iran is the second-largest pistachio producer worldwide after USA. Moreover, it was the first pistachio exporter in the past 5-years globally.

Contamination of pistachio nuts is a serious problem in terms of health and food security (Aminroosta *et al*., 2018). Various conditions such as ecological environment, farm, garden, seed, and soil management, soil moisture and air temperature, processing stage, and packaging may result in bacterial and fungal contaminations of nuts including pistachio (Demirci *et al*., 2012; Brown, 2018). The most frequent contaminating microorganisms isolated from pistachios are as follows: coliforms; *E. Coli*, salmonella; and several species of fungi, mainly *Aspergillus flavus* and *Aspergillus parasitiscus* producing aflatoxin (ISIRI15).

Finding a proper method to pasteurize raw pistachios while preserving the raw-like quality is a critical issue in the pistachio industry (Shaobo *et al*., 2007). In recent years, plasma sterilization has been emerging as an attractive

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alternative for chemical sterilization methods that are known for their intrinsic toxicity and leaving toxic residues on surfaces (Samuel *et al*., 1988). Several research works have identified the potential of non-thermal plasma technology in decontaminating pistachios. For example, Makari *et al.*(2021) used Dielectric Barrier Discharge (BDB) air plasma to eliminate *Aspergillus Flavus* from pistachio nuts, Esmaeili *et al.* (2021), used argon/air plasma jet to control *Plodia interpunstella* in pistachio, Pignata *et al.* (2014) used air/argon Plasma-Enhanced Chemical Vapor Deposition (PECVD) on raw, peeled, roasted, and salt free pistachios. Sohbatzadeh *et al.* (2016) used Dielectric Barrier Discharge (BDB) air plasma to inactivate *Aspergillus Flavus* spores in a sealed package. However, decontamination of pistachios has not been investigated by VNP, yet.

Plasma is the ionized gas consisting of a large number of different species such as electrons, positive and negative ions, free radicals, gas atoms, molecules in the ground or excited state, and quanta of electromagnetic radiation (photons). Notably, plasma sterilization is efficient with most gases such as O_2, N_2, air, H_2, halogens, N_2O_3, H_2O_2, CO_2, and SO_2, (Ratner *et al*., 1990). Depending on the type of energy supply and amount of energy transferred to the plasma, density and temperature of the electrons may change. These factors lead plasma to be distinguished into two categories, named as thermal plasma and non-thermal plasma (Nehra *et al*., 2008). A glow discharge is a kind of non-thermal plasma ionized gases that are generated under deep vacuum (low-pressure below 0.1 Torr) (Shintani *et al*., 2010).

The reactive spices in plasma have caused the oxidative effects on the outer surface of microbial cells. Accordingly, these act on the doubled bond of unsaturated fatty acid of membrane cells, thereby disturbing the transport of biomolecules across it (Guzel-Seydim *et al*., 2004). Despite oxidation of the lipids, amino acids and nucleic acids of cells and bacterial spores are vulnerable to the action of these spices. Moreover, the oxidation cause changes that lead to microbial death or injury (Critzer *et al*., 2007).

Regarding ISO 14937_2009_EN on the sterilization process, it is better to use high-resistance microorganisms that include a wide range of microorganisms ranging from gram-positive and gram-negative aerobic to anaerobic bacteria, mycobacteria, sporadic fungi, yeasts, parasites, and viruses.

G. stearothermophilus is one of the highest resistant bacteria, which belongs to *Bacillaceae* family, aerobic or facultative anaerobic bacteria, gram-positive, spore-forming, and thermophile bacteria with optimal growth between 55 °C and 65 °C (Nazina *et al*., 2001). *G. stearothermophilus* is a non-pathogenic bacterium that is responsible for 35% of canned food spoilage during incubation at 55°C (Ashton *et al*., 1992; André *et al*., 2013). Accordingly, it can be used as a Biological Indicator (BI) to examine the effectiveness of plasma for sterility assurance (Cheng *et al*., 2009; Guizelini *et al*., 2012; Rivero *et al*., 2012).

In this study, *G. stearothermophilus* was used as a Biological Indicator (BI) to measure the OD of VNP for inactivating bacteria. Thereafter, the effect of the obtained OD was examined on the microorganisms' population of pistachio samples to see a bacterial contamination reduction. Additionally, the physicochemical properties of the exposed pistachios were evaluated, and the assessed color, moisture, peroxide, and SEM were compared to untreated pistachios and the differences were indicated.

MATERIALS AND METHODS

First Step of Experiments

At the first stage, to test the effect of plasma on *G. stearothermophilus,* the obtained samples were examined using BIs.

Biological Indicator (BI)

A BI (gke, Germany) is a standard bacteria used to monitor the performance of sterilization. Following EN ISO 11138-6 reference for "Biological indicators for hydrogen peroxide vapor sterilization processes", used BIs need to contain spore of G. stearothermophilus bacteria with a definite number of microorganisms. Subsequent growth or failure of the

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microorganisms to grow under the defined conditions will indicate the adequacy of sterilization. In this experiment, the population of *G. stearothermophilus* spores on the disk examined was 10^6 CFU.

Using Glow Discharge Plasma (GDP) Device

A glow discharge is a type of VNP device that consists of a cylindrical Pyrex tube with 15 cm inner diameter. Two copper electrodes are connected around the body of the Pyrex and the voltage of the power supply is applied to them. With the help of a vacuum pump, the chamber pressure is adjusted in the range of 200 to 400 mm. The applied power is adjustable from 10 to 100 watts with a frequency of 20 kHz. A mass flow controller is used to adjust gas flow in the range ofStandard Cubic Centimeters per Minute SCCM (Figure 1).

The GDP process involves the following steps:

Vacuum step: Firstly, with the vacuum pump, it drains the air and other gases inside the chamber. Subsequently, this stage is a prerequisite for the next stage, and further reduction of pressure at this stage will help in better injection of the gas in the next step. This continues until the pressure has reached a relatively constant level.

Injection step: By turning on the generator, the specific gas will be injected into the chamber.

Figure 1. Glow discharge plasma device.

Diffusion step: The injected gas scatter in the chamber and the samples will be exposed to the gas.

Plasma step: By applying the voltage to electrodes, plasma was formed, which could deal with microorganisms and destroy them.

Aeration step: At the end of the process, hit the button of ventilation. The chamber is air-feed through an antibacterial filter, ventilated, and the pressure of the chamber reaches atmospheric pressure. Now, samples is brought out from device.

Treatment of BIs

At first, BIs were exposed to the plasma in the flow of 3 SCCM, primary pressure of 400 mTorr, at different times and with different powers (Figure 2).

Microbial Analyses of BIs

In terms of the gke protocol, the strip of bacteria was aseptically transferred into a sterile blender cup containing 10 mL chilled processed water, and homogenized 3-5 min until no large particles were visible. Following US Pharmacopoeia, it was recommended to heat shock tube in a water bath for 15 min at 95-100ºC. Afterward, immediately, tubes were cooled in a water bath at 0 – 4ºC. Then, serial dilution was performed and pour plate was prepared on

Figure 2. BI put into VNP.

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TSA (Tryptic Soy Agar) culture. Finally, it was incubated for 48 hours at 55-60°C and the number of Colonies Forming Units (CFU's) per plate was recorded.

Second Step of Experiments

Raw pistachios, Akbari brand, were purchased locally from nuts store specialist for pistachios in Tehran. Then, they were stored under refrigerated conditions (4-8ºC) in the aseptic condition so that the microbial load does not change.

Firstly, the pistachios were unshelled aseptically, and then, 10 g of unshelled pistachios were transferred to a sterile glass dish under aseptic condition, and were exposed to the plasma.

Plasma Treatment

Plasma conditions were a flow of 3 SCCM, primary pressure as 400 mTorr, in a different time and with different powers using two gas of oxygen and air (Figure 3).

Microbial Analyses of Pistachios

In reference to the Iranian national standards organization (INSO) 8923-4 and 9899 and national standard ISIRI 8923-1, the treated pistachios were transferred to 90 mL of Peptone Water (PW) and mixed using a homogenizer device. Subsequently, the pistachios were flattened out with filter paper, so, the initial suspension was prepared. Then, dilution series were prepared and regarding ISIRI 5272, Plate Count Agar (PCA) culture was used to make pour plate culture, which was then incubated for 72 hours at 30 ºC. After the incubation, colony count was performed in terms of the ISIRI 5272.

FLIR (Forward Looking Infrared)

The FLIR E4 device works with infrared radiation. Immediately after removing the samples from the plasma chamber, the FLIR was used to measure the temperature of the samples. The reason for performing this test was to control the samples temperature that microorganisms death did not occur due to high temperature.

Physicochemical Changes

In this study, changes in color parameters due to plasma exposure were designated as another specification to be considered for nutrients of the products.

The color model Lab is often used for research on food by using hunter lab devices. According to the Commission Internationale de l' Eclairage (CIE), Lab model recognized as an international standard for the evaluation of the color changes, allows for a color specification in 3-D space (Yang *et al*., 2017). Expressing color values as L* (Whiteness/Darkness), a* (redness/greenness), and b*

Figure 3. Pistachio sample put into VNP.

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(yellowness/blueness) allowed us to calculate the color difference (ΔE) in the pistachio samples after treatment (Lei Xu *et al*., 2017).

$$\Delta E = [(l_{standard}-l_{sample})^2+(a_{standard}-a_{sample})^2+(b_{standard}-b_{sample})^2]^{0.5} \quad (1)$$

Where, …please define the symbols.

Determination of Moisture

To test the moisture content of the pistachios after the plasma treatment, a direct drying method was applied in terms of the INSO 672. 10g of the pistachios was weighed, placed in a dried glass jar, and then kept for at least 3 hours in the drying oven at 105ºC. Next, they were allowed to cool down to room temperature in desiccators for half an hour, then, weighed. The difference between the first and final weight indicated the moisture of pistachios.

Determination of Peroxide

To determine peroxide in the pistachios, according to ISIRI 4179, 100 g crushed pistachios with 150-200 mL petroleum ether at the boiling point of 40-60ºC were put on Erlenmeyer. Afterward, the door of Erlenmeyer was closed and kept in the hood for 24 hours. Thereafter, using Soxhlet extractor, petroleum ether was evaporated, so, oil was extracted. Next, we added acetic acid, chloroform, and iodoral potassium. Then, added 25 mL distilled water and continued titration with sodium thiosulfate 0.02N until disappearance of the blue color in the solution. Finally, we measured the peroxide number according to mili-equivalent gram (Meq O_2/g):

Peroxide number= Sodium thiosulfate (mL)×Sodium normality×1000/Weight of pistachio (g) (2)

Index of peroxide is shown by Meq O_2/g peroxide or active oxygen in a kilogram of oils or fats.

Scanning Electron Microscopy (SEM)

Evaluation of the changes in surface of the pistachios after the VNOP treatment was performed using SEM (Hitachi SU3500) compared with the untreated samples. Small square pieces (approximately 1 cm per side) of 2 pistachios were cut and placed in sterile petri dishes. Thereafter, they were coated with a thin layer of gold and the samples were observed in SEM.

Optical Emission Spectroscopy (OES)

Optical Emission Spectroscopy (OES) was performed to characterize the reactive gas species generated in plasma during the treatment (Moiseev *et al*., 2014). In this method, plasma is transmitted through the optical fiber to the spectrometer, then, it would be analyzed. OES was measured using an Avantes (AvaSpec-3648-USB2) spectrometer with a wavelength range from 200 nm to 1,100 nm. Note that the distance between the optical fiber and the plasma chamber was 300 mm.

Statistical Analysis

Analysis of variance and significant differences among the means were tested by one-way ANOVA and independent sample T-student test using SPSS software by applying LSD-DUNKAN test in Error level at 95% ($P< 0.05$), respectively (Version 19.0 for windows, SPSS).

RESULTS AND DISCUSSION

Effect of VNAP on G. *stearothermophilus*

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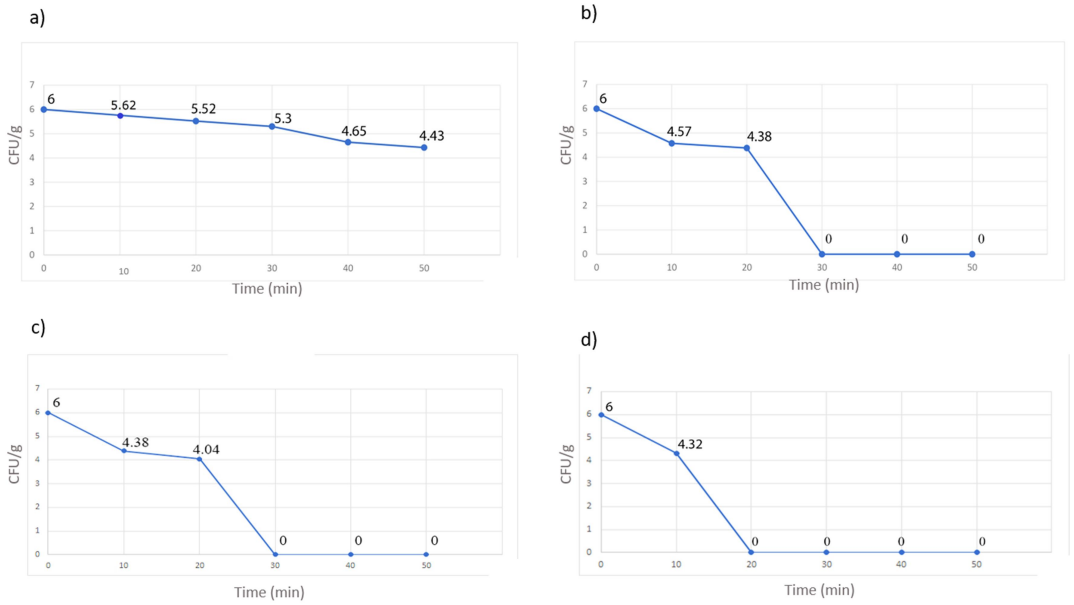

Figure 4. Effect of VNAP on *G. stearothermophilus* at: (a) 30, (b) 50, (c) 70, and (d) 90W and different times.

To evaluate the effect of VNAP on *G. stearothermophilus,* the differences among various groups were examined using a one-way ANOVA statistical method at the confidence level of 95%. The significant effect of VNAP on *G. stearothermophilus* bacteria between the treated and untreated samples is shown in Figure 4. According to *p-value* that became zero, it can be concluded that total population of surface bacteria decreased, while power and time increased. Thereafter, using LSD-DUNKAN test at the confidence level of 95%, it was shown that the untreated samples had significant differences with all the treated ones. In low doses of power, like W= 30, in different times of 10, 20, 30, 40, and 50 minutes bacterial load decreased while the time increased. However, when W= 50, in the times of 10 and 20 minutes, the bacterial log decreased but did not reach zero. (Table 1). The power of 50W in 30 minutes was the lowest power through which bacterial log became zero. Then, by increasing time, power, or both, in the power of 70W, log reduction became zero when time was more than 30 minutes. The next zero dose was for 90W after 20 minutes, in which time reduced, but plasma dose increased, which may cause some side effects on the samples. The first and the best log reduction occurred in power of 50W and in 30 minutes. So, this is considered as the OD that is the most appropriate reduction of bacterial load compared to the other time and powers.

Table1. Effect of VNAP *G. stearothermophilus* at different powers and times.

Power (W)	Time (Min)	*G. stearothermophilus* (CFU)
0	0	10^6
30	10	4.2×10^5
30	20	3.3×10^5
30	30	2×10^5
30	40	4.5×10^4
30	50	2.7×10^4
50	10	3.7×10^4
50	20	2.4×10^4
50	30	0
50	40	0
50	50	0
70	10	2.4×10^4
70	20	1.1×10^4
70	30	0
70	40	0
70	50	0
90	10	2.1×10^4
90	20	0
90	30	0
90	40	0
90	50	0

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Effect of VNAP on Pistachios

To investigate the effect of VNAP on pistachios' microbial contamination, the results of experiments accessed from *G. stearothermophilus* bacteria, were patterned. Firstly, the power of 50W at 30 minutes was examined. But microbial load did not become zero and 1.0 log reduction was observed, which showed a significant difference. For 50W, by increasing time of exposure (to 50, 70, and 90 minutes), microbial load decreased 2.0 log, but it did not reach zero (Table 2) (Figure 5).

By increasing power to 70W, 2.0 log microbial load reduction was observed. In further doses, the temperature increased that, causing the error in results, using the power of 70W for 30 minutes resulted in more microbial population reduction, but temperature of the samples dramatically increased up to nearly 70ºC, which could affect morphology and quality of pistachios or any other similar food. (Figure 6).

Effect of VNOP on Pistachios

The results obtained from VNAP were used as follows:

Using VNOP, more log reductions were observed. In power of 50W and times of 30, 50, 70, and 90 minutes, 2.0 log reduction was observed with a significant difference (Figure 7) (Table 3).

Comparison of VNAP and VNOP on Pistachios' Microbial Contamination:

One-way ANOVA statistical method, LSD-DUNKAN test, and t-test at the confidence level of 95% showed that the untreated pistachio samples had significant differences with the treated groups. The

Table 2. Effect of VNAP on pistachios at different powers and times.

Power (W)	Time (Min)	Pistachios microorganisms (CFU)
0	0	3×10^4
50	30	5.5×10^2
50	50	4.3×10^2
50	70	1.6×10^2
50	90	1.2×10^2

Table 3. Effect of VNOP on pistachios at 50 W and different times.

Power (W)	Time (Min)	Pistachios microorganisms (CFU)
0	0	3×10^4
50	30	1.1×10^3
50	50	4.4×10^2
50	70	1.8×10^2
50	90	1.4×10^2
70	30	3.4×10^2

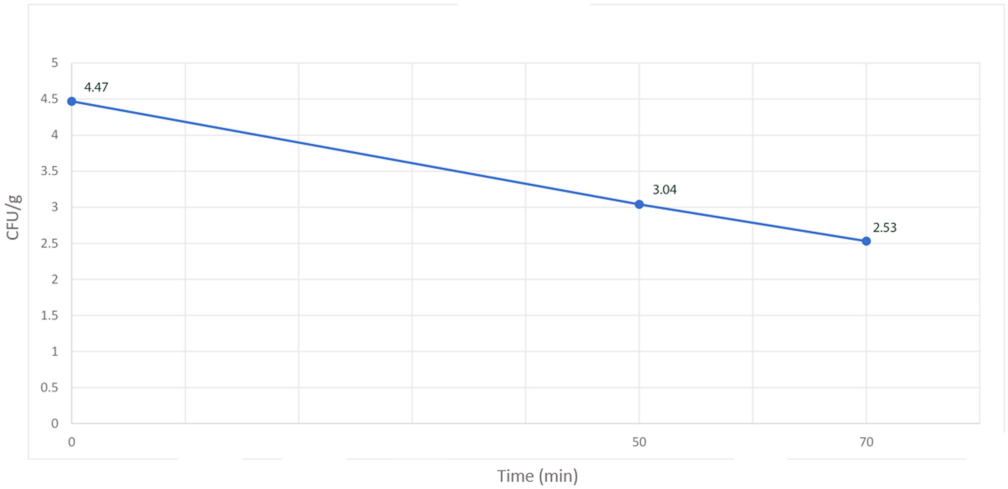

Figure 5. Effect of VNAP on pistachios at 50W and different times.

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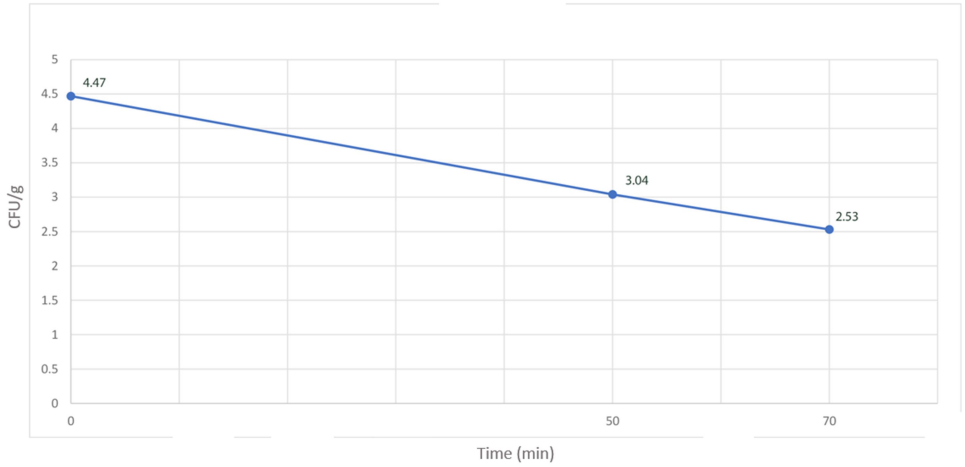

Figure 6. Effect of VNAP on pistachios in the time of 30 minutes and powers of 50 and 70W.

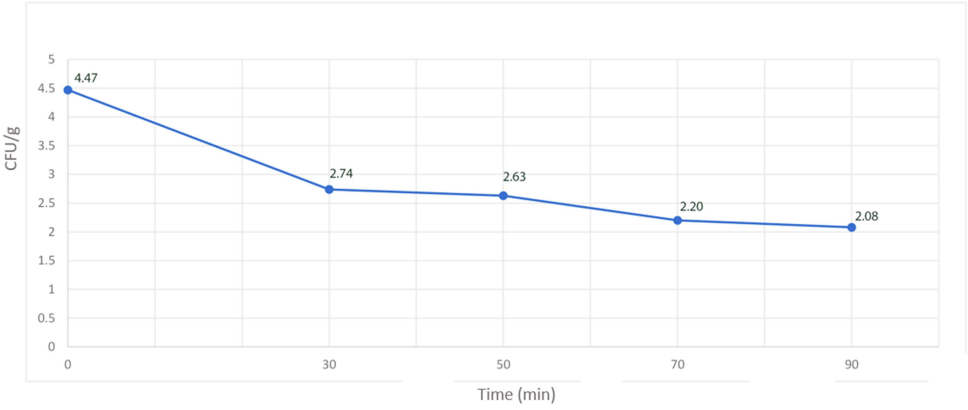

Figure 7. Effect of VNOP on pistachios in the power of 50W and different times.

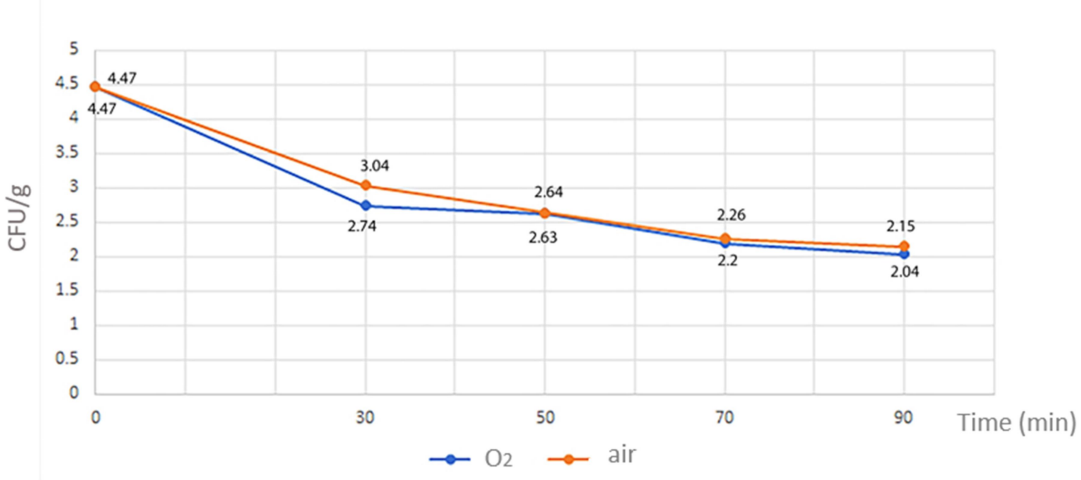

Figure 8. Comparison of VNAP and VNOP on pistachios at 50W and different times.

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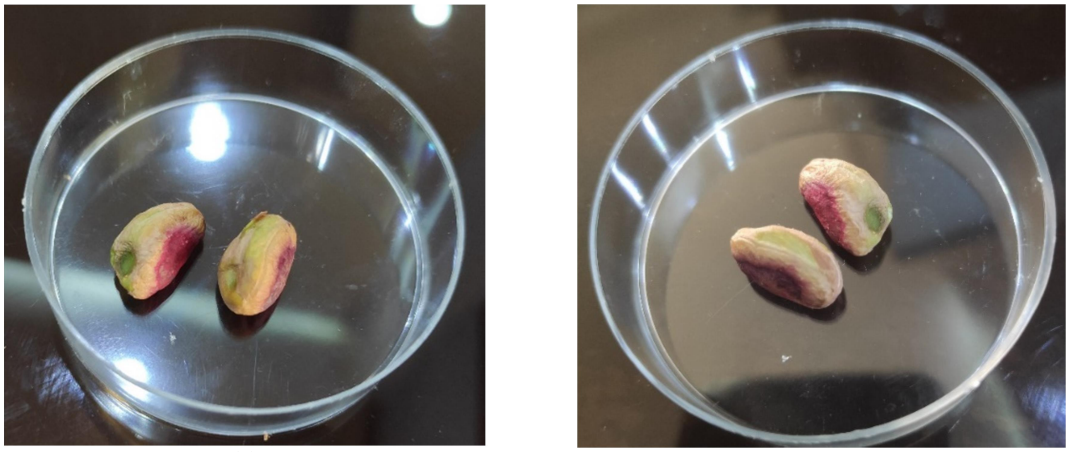

(a) (b)

Figure 9. Pistachios sample (a) before treatment, (b) after treatment with VNOP.

Table 4. The CIE L*-a*-b* values of the control and plasma treatments.

ΔE	b∗	a∗	L∗	Sample
00.00±00.00	18.55±0.07	10.6±0.11	42.77±0.01	Control
3.03±0.02	19.27±0.01	10.91±0.09	45.7±0.02	Plasma treatment

pistachios samples exposed to VNOP in 50W after 30 minutes showed 2.0 log microbial reduction, while the samples exposed to VNAP showed 2.0 log reduction after 30 minutes at 70W (Figure 8). Microbial load in the power of 50 W VNOP in 30 minutes is the same as the power of 50W VNAP in 50 minutes, and the best OD was in power of 50W VNOP in 30 minutes. So, it can be said that VNOP is more effective on pistachios than VNAP, but the difference is not significant.

Distribution of OD

According to the obtained results and the parameters tested, optimum time and power obtained in 50W and 30 minutes are the most appropriate reductions observed in microbial load, compared to other time and powers. In Figure 5, the reductions of microbial load in air and oxygen plasma are compared. No significant differences were observed in microbial reduction of air plasma and oxygen plasma. Since the microbial load is less in oxygen plasma, it can be chosen as the optimum level of an effective plasma.

Physicochemical Changes

The plasma-exposed pistachio samples given under the optimal conditions were examined.

The apparent changes in pistachios properties, i.e. color, moisture, etc. were also measured. The color of the pistachios were not altered in comparison with the control samples, as both samples were pink/green (Figure 9).

Colorimetric Results

Evaluation of the surface color parameters of the pistachios revealed that plasma treatment affected the Lightness (L∗), but did not affect contrast (a∗) and the

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blue/yellow contrast (b∗), as shown in detail in Table4. According to ISIRI 20747:

If ΔE< 1.5 small differences

If 1.5< ΔE< 3 distinct differences

If ΔE> 3 very distinct differences

The ΔE∗ value of the pistachios significantly changed (Table 4).

The colorimetric results ΔE≈3 showed some significant changes in the specimen. Using LSD-DUNKAN test at the confidence level of 95%, it was shown that the untreated samples had significant differences in L* with all the treated ones, which can significantly influence the lightness of sample, but they are not distinguishable with naked eyes.

Determination of the Extracted Fat Peroxide

As shown in Table 5, no changes were found in fat peroxide at all.

Determination of Humidity

The results of humidity measurement between plasma treatments and the control samples demonstrated no significant difference (Table 6). The amount of moisture decreased in pistachios, which was a useful reduction after the treatment because it increased the immunity of pistachios from post-plasma environmental pollution.

OES

OES was used to identify the major active spices generated O_2 and air plasma discharge. OES was also used to analyze the intensities of ions, radicals, and active species produced during the plasma treatment. The intense peaks in spectra correspond to the emissions in the near UV region by the excited species of nitrogen, namely, nitrogen second positive system N_2, and the first negative system N_2^+ that were obtained via the emission of air plasma (Filatova *et al*., 2013) (Figure 10-a).

The main excited species were atomic oxygen O (777, 844 nm) and molecular oxygen ions O2+ (599 nm) that were obtained via the emission spectrum of oxygen plasma (Wan *et al*., 2017) (Figure 10-b). Notably, these excited atoms, radicals, active species, and ions can interact with microorganisms in many different ways such as physical bombardment of cell wall of the microorganisms by energetic ions, chemical interaction with active species that oxidize the cells, and Ultraviolet (UV) disinfection of microorganisms. It was shown which ions and active radicals were the most frequent types, possibly decontaminating the microorganisms. Moreover, the effects of the active by-products of non-thermal plasma were analyzed through tissue's examinations in terms of structure and chemical compounds (Hosseini *et al*., 2018).

Table 5. The extracted fat peroxide of the control and plasma treatment samples of pistachios in the optimum point.

Limit	Plasma treatment	Control	
1>	0.1	0.1	Extracted fat peroxide (Meq_{O2} kg^{-1})

Table 6. Humidity changes in plasma treatment pistachios compared with the untreated pistachios.

Limit	Plasma treatment	Control	
5>	2.3±0.02	3.1±0.01	Humidity (g%)

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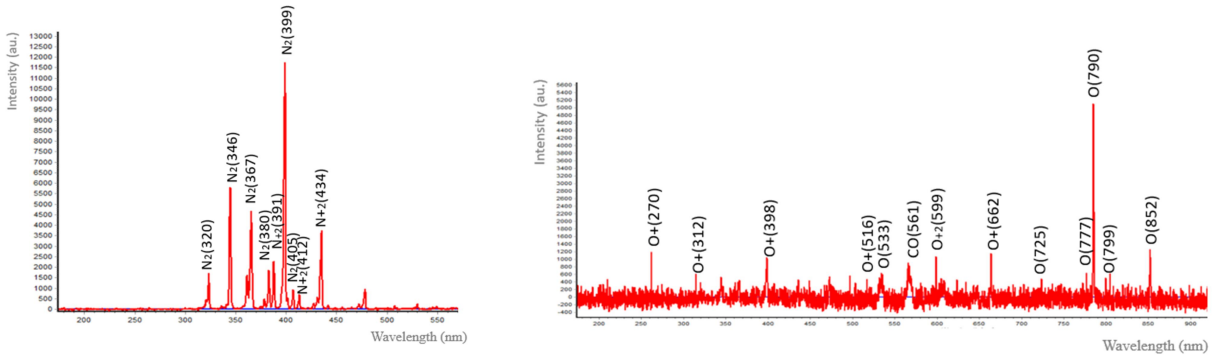
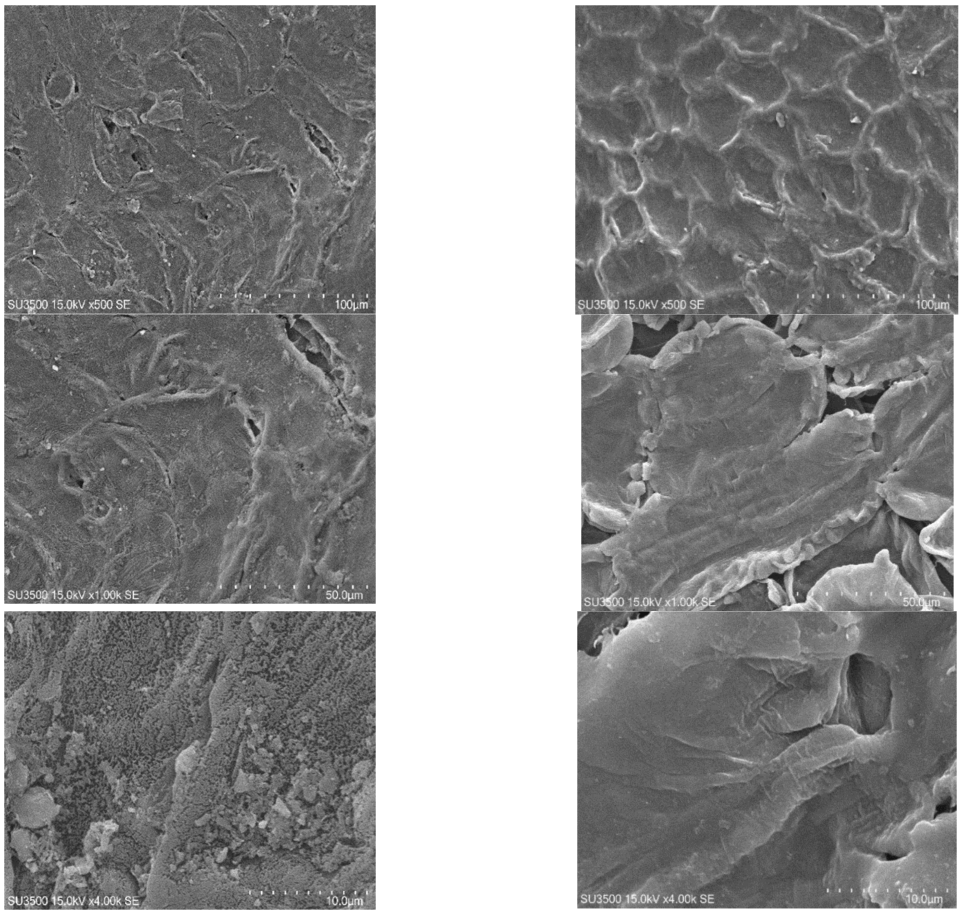


(a) (b)

Figure 10. OES results of VNP in (a) VNAP and (b) VNOP.



(a) (b)

Figure 11. SEM of pistachios with VNOP by zoom in 500, 1000 and 4000 in (a) The control samples and (b) The treatment samples.

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Surface Morphology

Since the colorimetric results, ΔE≈3 showed some significant changes in the specimen, but they are not distinguishable with naked eyes, but SEM used showed the effect on pistachios' appearance.

SEM was used to study the morphological changes of the pistachios following the optimum point by VNOP treatment. By comparing the plasma-treated pistachios with the control pistachios in Figure 11, it was observed that, after the treatment, the pistachios' surface was smoother than before; in a way that the surface of the control pistachios was sliced and reticulated, but the treated pistachios' surface was trimmed and smoothed. In addition, only slight protrusions were seen in some places where this deformation is justified by the effect of plasma on surface of the pistachios resulting from bombardment of it by charged particles and radicals produced by plasma. It indicated that there was a direct relationship between the lethal velocity and the topology of the sample's surface.

CONCLUSIONS

Overall, the results show that the total population of microorganisms decreased along with increasing power and time in VNP equipped with both oxygen gas and air. Therefore, the higher the plasma power and the longer the exposure time of the pistachio samples, the higher the reduction in the microbial load. By increasing the plasma irradiation time of the specimens, due to the increased activity of plasma-producing charged particles, the surface microbial degradation raised. Moreover, in the case of oxygen gas, the rate of microbial charge reduction was more effective than air, because of the presence of more radicals, while cell death was due to O_2 oxidation.

Consequently, the success of VNP proved to cause an acceptable reduction of pistachio-contaminated microorganisms with no significant difference in physicochemical properties, so, it can totally inactivate bacterial contamination.

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بررسی پلاسمای تحت خلاء در غیر فعال سازی میکروارگانیسم های پسته: مطالعه فیزیکی و شیمیایی

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چکیده

این مطالعه با هدف بررسی تاثیر پلاسمای غیر حرارتی تحت خلا بر رفع الودگی سطحی پسته انجام شد. ایمنی مواد غذایی به عنوان یک مسئله مهم در صنایع غذایی در نظر گرفته می شود و سم زدایی یک گام ضروری در طول پردازش مواد غذایی است. پلاسمای غیر حرارتی خلا به طور گسترده ای به عنوان یک روش موثر در ضدعفونی کردن مواد غذایی و پردازش مواد غذایی در نظر گرفته شده است. در این ازمایش، توانایی پلاسمای غیر حرارتی تحت خلا برای غیرفعال کردن باکتری های شاخص بسیار مقاوم Geobacillus stearothermophilus میکروارگانیسم های الوده به پسته مورد بررسی قرار گرفت. در مرحله اول، اثر پلاسمای هوای غیر حرارتی تحت خلاء (VNAP) بر G. stearothermophilus در توان ۳۰، ۵۰، ۷۰، ۹۰ وات و ۱۰، ۲۰، ۳۰، ۴۰، ۵۰ دقیقه بررسی شد. نتایج نشان می دهد حداقل دوز پلاسمای هوای غیرحرارتی تحت خلا برای غیرفعال کردن باکتری G.stearothermophilus به طورکامل توان ۵۰ وات در ۳۰ دقیقه است. بنابراین، می توان آن را به عنوان یک دوز بهینه (OD) برای درمان پلاسمای هوای غیر حرارتی خلاء در نظر گرفت. پس از ان، از دوز بهینه برای بررسی اثرات پلاسمای هوای غیر حرارتی تحت خلاء و سپس پلاسمای اکسیژن غیر حرارتی تحت خلاء (VNOP) بر میکروارگانیسم های آلوده کننده پسته استفاده شد. نمونه های پسته در معرض پلاسمای هوای غیر حرارتی تحت خلاء در دوز بهینه (۵۰ وات برای ۳۰ دقیقه) کاهش بارمیکروبی به میزان ۱ لگاریتم را نشان دادند. هر دو پلاسمای هوا و اکسیژن غیر حرارتی تحت خلاء بار میکروبی پسته را به طور قابل توجهی کاهش دادند، اما پلاسمای اکسیژن غیر حرارتی تحت خلاء موثرتر از پلاسمای هوای غیر حرارتی تحت خلاء بود. بنابراین پلاسمای اکسیژن غیر حرارتی تحت خلاء در ۵۰ وات به مدت ۳۰ دقیقه می تواند به عنوان دوز مطلوب انتخاب شود. نمونه های پسته نیز با استفاده از خواص فیزیکوشیمیایی در دوز بهینه مورد تجزیه و تحلیل قرار گرفت. در نتیجه، پلاسمای غیر حرارتی تحت خلا در ضدعفونی پسته موثر بود و هیچ تاثیر معنی داری بر خواص ان نداشت.

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