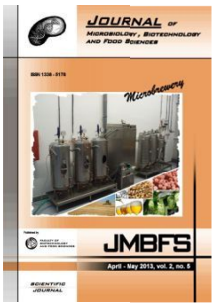

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EFFECT OF PLASMA ENERGY ON THE ANTIOXIDANT ACTIVITY, TOTAL POLYPHENOLS AND FUNGAL VIABILITY IN CHAMOMILE (*MATRICARIA CHAMOMILLA*) AND CINNAMON (*CINNAMOMUM ZEYLANICUM*)

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ABSTRACT

The plasma energy is a collection of free particles with positive and negative charges and has demonstrated to be a good prospect for food preservation. The aim of this study was to evaluate the effect of plasma energy on the antioxidant activity, total polyphenol content and yeasts and molds counts in chamomile and cinnamon powder samples treated at 650, 750 and 850 volts for 0, 1, 3 ,5 7 and10 min. Total counts of yeasts and molds in chamomile and cinamon powder samples were determined before and after each treatment with plasma energy. The enumeration was conducted on potatoe dextrose agar suplemented with 0.6% Bengal rose and 2% ampicillin. The antioxidant activity and total polyphenol content were also analyzed. The results showed that plasma exposure on chamomile and cinnamon powder at 850V for 10 minutes significantly reduced ($p<0.05$) the concentration of yeasts and molds reduced to <1.0 log CFU/g, and only 0.68±0.19 log CFU/g, respectively. Regarding the antioxidant activity and the total polyphenol content, we observed a reduction of 55% in the antioxidant activity in chamomile, while in cinnamon; there was an increase of 21.4% at 750 V. The highest total polyphenol content was observed after 10 min of treatment at 650 V and 750V, with concentrations of 3.3 ± 0.05 mg GAE/g in chamomile and 1.7 ± 0.01 mg GAE/g in cinnamon. We conclude that treatment with plasma at 750 Volts and 10 minutes of exposure was the best treatment to significantly reduce ($p<0.05$) yeasts and molds counts without affecting the total polyphenol content in chamomile and cinnamon powder.

Keywords: Plasma, antioxidants, phenolic content, yeasts and molds, food safety

INTRODUCTION

Several studies have been carried out by groups of researchers specialized in the generation, characterization and application of non-thermal atmospheric pressure plasma, particularly in the biomedical area, producing remarkable studies on methods and mechanisms of bacterial inhibition and sterilization (**Peña-Eguiluz, *et al*., 2010a; Moisan *et al*., 2002**). The system has demonstrated the possibility of application on organic materials due to its non-aggressive nature (**Stoffels, *et al*., 2002**).

It has been shown in recent years, that plasmas generated outside thermal equilibrium at atmospheric pressure produce an antimicrobial effect, (**Laroussi, 2000; Abramzon *et al*., 2006; Roth *et al*., 2007; Kirkpatrick *et al*., 2007; Peña-Eguiluz, *et al*., 2010b**), resulting from the interaction of the organic media with a wide variety of active oxidizing species , excited atoms and molecules, as well as ultraviolet radiation that are produced during plasma interaction with air. Some studies have shown that use of plasma may have numerous applications in medical, biological and environmental areas, mainly because it is a non-thermal process at atmospheric pressure (**Moisan *et al*., 2002**; **Stoffels, *et al*., 2002; Abramzon *et al*., 2006; Gallagher *et al*., 2007**). This process has been reported as a successful tool for the inactivation of microorganisms, including Gram-negative and Gram-positive bacteria exposed to plasma on flat surfaces, raising an interesting alternative for the inactivation of microorganisms in the food industry (**Laroussi, 2000**; **Gallagher *et al*., 2007**).

Although there is information regarding the use of plasma on the inactivation of certain microorganisms, this technology still requires further studies to standardize the method and conditions necessary to inhibit a larger number of microorganisms (**Yan *et al*., 2010; Vrajova *et al*., 2009**). The Conditions for the inactivation of diverse types of microorganisms by plasma application should be different, particularly when the treatment is applied to inactivate those with ability to survive harsh conditions such as sporulated and encapsulated microorganisms (**Laroussi 2002**).

An important problem for the food industry is the application of severe sterilization processes that reduce the nutritional quality of their products, usually is lost due to severe sterilization process or by microbial contamination, which may cause physical and nutritional deterioration. The use of plasma could be a good alternative for food preservation because does not generate heat that might reduce the nutritional components, but reduce microbial populations avoiding product deterioration (**Perni *et al*., 2008; Grzegorzewski *et al*., 2009**).
There is little information on the effects of plasma on nutritional and functional components of foods (**Perni *et al*., 2008**). **Grzegorzewski *et al*. (2009)**, studied the degradation of different phenolic compounds (quercetin, myricetin, kaempferol and taxifolin) after application of argon plasma at 10 to 30 W of power and a flow of 20 standard cubic centimeters per minute (SCCM). Results showed that plasma causes degradation of these compounds reflected by the antioxidant activity loss and a reduction of 90% of the antioxidant activity of quercetin and kaempferol was reported.

Cinnamon and chamomile are spices and herbs used worldwide; they are used to prepare tea and because they contain flavonoids and polyphenolic compounds with antioxidant activity its infusions have various medical applications (**Bakkali *et al*., 2008; Loranty *et al*., 2010**). The presence of fungi in dry foods such as chamomile and cinnamon is related to the exposure of these products to the environment and to deficiencies associated with poor management practices during storage, causing product deterioration and losses.

The aim of this paper is to demonstrate the ability of the dielectric barrier plasma produced at atmospheric pressure to reduce the number of yeasts and molds in chamomile and cinnamon powder samples, as well as to study its effect on the total polyphenol content and antioxidant activity to provide a possible alternative for plasma application in the food industry.

MATERIAL AND METHODS

Food samples

Chamomile (*Matricaria chamomilla*) and cinnamon (*Cinnamomum zeylanicum*) produced in the region of Jalisco State, Mexico, were used for this study. Three kilograms of every sample were obtained in bulk at Corona market in June. They were transported in bags of polyethylene. And in the laboratory, the samples were homogenized by means of manual agitations. They were then

packaged and sealed in new bags of polyethylene and stored up to his analyses. Every bag contained approximately 250 g.

Plasma generator

The plasma generator is formed by dielectric barrier discharge (DBD) and radio frequency (RF). It was designed and built at the Laboratory of Plasma Physics of the National Nuclear Research Institute in Toluca, Mexico. The device comprises a power source of 13.56 MHz and a RF RDBD constituted by a pair of parallel electrodes in a stainless steel circular cylinder contained in a Pyrex ® glass. One of the electrodes can be moved to adjust the distance between plates. The upper tubular shaft allows gas application directly to the sample creating an electrical discharge between both electrodes.

Treatment of cinnamon and chamomile powder with plasma energy

Chamomile and cinnamon powder samples of 1 g were placed on plate petri, they had approximately 1 mm of thickness and then treated with plasma energy at atmospheric pressure. Plasma was generated using a commercial helium gas (Praxair, Mexico) at a constant flow of 1.5 L/min and applied at different time intervals (0, 1, 3, 5, 7 and 10 min) and at 650, 750 and 850 Volts. Before and after each plasma treatment, the enumeration of yeasts and molds (log CFU/g) , as well as antioxidant activity and total polyphenol content were determined . Each experiment was conducted in triplicate.

Antioxidant activity

Chamomile and cinnamon powder samples treated with plasma energy were prepared as an infusion (1 g/10 mL water), to determine the antioxidant activity by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method of radical scavenging with some modifications (**Brand-Williams *et al.*, 1995**) and to analyze for total polyphenol content (**Price and Butler, 1977**). A 50 µL aliquot of the chamomile or cinnamon infusion was placed in a test tube and 950 µL of a 100 µM methanolic (Sigma Aldrich, Germany) solution of DPPH radical (Analytical Fluka, Germany) were added. Absorbance measurements were immediately conducted using a spectrophotometer Genesis 20 (Thermo spectronic, United States) at 517 nm every 30 s during 10 min. Results were expressed as IC50 which indicates the concentration required to inhibit 50% of the free radical, and was determined by regression analysis of percent inhibition *versus* concentration required of the aqueous extracts to inhibit 50% of DPPH radical (**Katalinic *et al.*, 2006**).

The percentage of inhibition of DPPH radical was calculated as follows:
Equation 1: % Inhibition DPPH = (DPPH) i-(DPPH) f / (DPPH) I *100

Where:
(DPPH) **f,** is the absorbance of DPPH radical at the end of the reaction.
(DPPH) **I,** is the absorbance at the start of the radical reaction.

Determination of total polyphenols content

The total polyphenols content was performed using the Prussian blue method (**Price and Butler, 1977**). An aliquot of 400 L of chamomile or cinnamon infusion were added with 1.2 mL of 0.1M FeCl3 (Caledon, Canada) in 0.1N HCl (Panreac, Spain) and 1.2 mL of 0.008M of K3Fe(CN)6 (Caledon, Canada). After 10 min of reaction, the absorbance at 720 nm was measured with a spectrophotometer Genesis 20 (Thermo spectronic, United States). This test was performed with three repetitions per sample. The results were expressed as mg of Gallic acid equivalents (GAE)/g of dry sample.

Enumeration of yeasts and molds

Yeasts and mold enumeration was performed using the **NOM-111-SSA1-1995** method. A 1 g aliquot of chamomile or cinnamon sample was diluted in 90 mL of 0.1% peptone water and homogenized for 1 min in a peristaltic blender (Stomacher-400, Seward, UK). Decimal dilutions were performed and pour plated with potato dextrose agar (PDA, Bioxon, Mexico) containing 2% ampicillin (Bayer, México) and 0.6% Bengal rose (Analytyka, Mexico). All plates were incubated at 20°C for 120 h. Yeasts and molds colonies were enumerated and results reported in log CFU/g of dry sample (**Vanderzant and Splittstoesser, 1992**).

Decimal reduction time (D-value)

The decimal reduction time (D-value), considered as the time required to inactivate 90% of the microbial population in logarithmic scale (**Pflug *et al.*, 2000**), was calculated for the yeasts and molds population from the slope of the inactivation plot obtained after plasma treatments were applied at different times and voltages, the results were obtained using Microsoft excel.
Equation 2:

Where:
N: log CFU/g of initial sample
No: log CFU/g of sample end
T: Time of treatment.

Statistical Analysis

Statistical analysis was performed using STATGRAPHICS Centurion XV software version 2.15.06. Analysis of variance (ANOVA) and multiple comparison procedures (Least Significant Difference–LSD) test were conducted to determine whether there were significant differences ($p<0.05$) among treatments.

RESULTS AND DISCUSSION

Antioxidant activity in chamomile and cinnamon powder treated with plasma energy

The radical DPPH is useful to evaluate the antioxidant activity of free polyphenol radicals and low IC50 values reflect high scavenger activity of free radicals (**De Ancos *et al.*, 2000**). After 10 min of treatment, the antioxidant activity in chamomile treated with plasma energy (Fig. 1) indicates a significant loss of antioxidant activity when the product was treated at 750V (51%) and at 850V (55%) ($p<0.05$), while at 650V no significant changes were observed after 10 min of treatment ($p<0.05$).

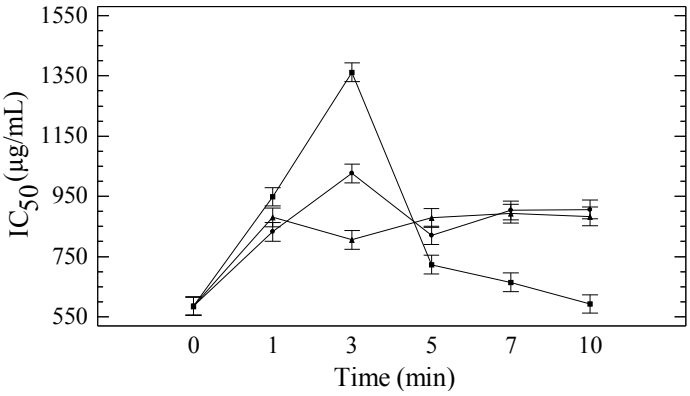

Figure 1 Antioxidant activity (expressed as IC50) in chamomile (*Matricaria chamomilla*) samples treated with plasma at 650 (▪), 750 (▲) and 850 (●) Volts

Regarding the antioxidant activity in cinnamon samples treated with plasma energy (Fig. 2), an increase in antioxidant activity was observed, which was measured according to the IC50 value at 750 V (21.4%), 850 V (12.2%) and 650 V (14.4%) after 10 min. We assume that total polyphenols content has differences between antioxidant activities, expressed as IC50, in the samples of chamomile and cinnamon. It is known that polyphenols antioxidant properties vary according to their chemical structure, (**Vajragupta *et al.*, 2000; Van Acker *et al.*, 1996**) the provision of functional OH groups (**Trouillas *et al.*, 2006**) and activation parameters as enthalpy, which influence the ability of polyphenols to scavenge free radicals, diminishing their biological activity (**Senba *et al.*, 1999; Ray *et al.*, 2007**).

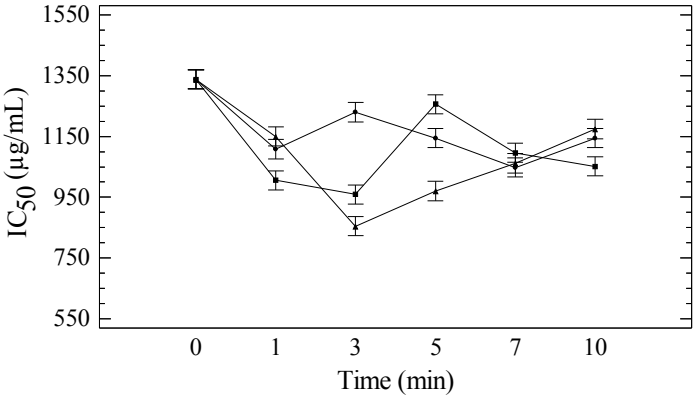

Figure 2 Antioxidant activity (expressed as IC50) in cinnamon (*Cinnamomum zeylanicum*) samples treated with plasma at 650 (▪), 750 (▲) and 850 (●) Volts

Total polyphenols content

The total polyphenols content in chamomile samples (Fig. 3), showed significant variations ($p < 0.05$). The treatments at 650 and 850 volts showed positive effects, while at 750 volts presented negative effects. The total polyphenol content, at the start was 2.77 ±0.04 mg GAE/g dry chamomile, decreasing 1.57±0.05 mg GAE/g dry chamomile in 38 % ($p < 0.05$) after treatment with plasma energy at 750 V during 10 min. Furthermore, it was observed that at 650 and 850 V there was an increase in total polyphenols content of 17.7 and 5.4%, respectively. The highest total polyphenol content was observed after 10 min of treatment at 650 V obtaining 3.3±0.05 mg GAE/g of chamomile.

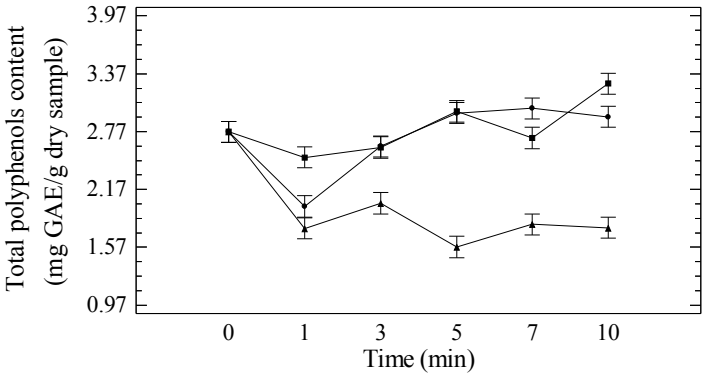

Figure 3 Total polyphenol content in chamomile (*Matricaria chamomilla*) samples treated with plasma at 650 (▪), 750 (▲) and 850 (●) Volts

The results of total polyphenols quantification in cinnamon (Fig. 4) showed that treatments at 750 and 850 volts, showed positive effects, while at 650 volts presented negative effects. The treatment at 650 V caused a significant decrease of total polyphenol content in 33.1% ($p < 0.05$), whereas at 750 and 850 V, an increase of 3.9% and 13% was observed, respectively. The highest total polyphenol content was observed after 10 min of treatment at 750 V obtaining 1.7 ±0.01 mg GAE/g of cinnamon.

Polyphenols are secondary metabolites found in plants and characterized by having beneficial effects to health through different action mechanisms (**Hervert-Hernández *et al*., 2011; Faller and Fialho, 2010**), however, they are sensitive to various physicochemical factors, like heat treatment, ionizing radiation, UV light, showing variations in chemical structure that causes these having different molecular properties (**Vajragupta *et al*., 2000; Van Acker *et al*., 1996**).

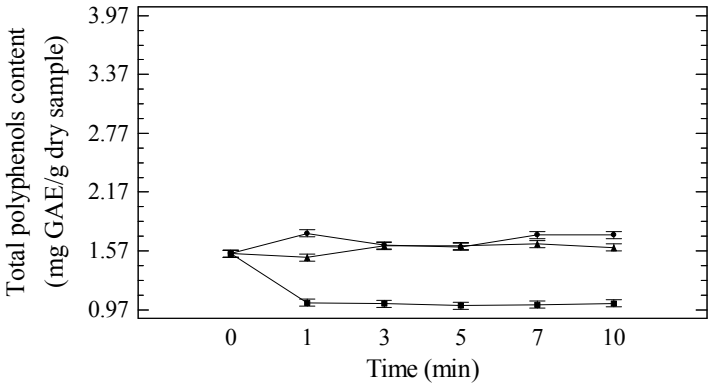

Figure 4 Total polyphenol content in cinnamon (*Cinnamomum zeylanicum*) samples treated with plasma at 650 (▪), 750 (▲) and 850 (●) Volts

Grzegorzewski *et al.* (2009), studied the effect of plasma energy using argon gas, on purified polyphenolic compounds including quercetin, kaempferol, taxifolin and miricietin dissolved in 50% methanol for 120 seconds of treatment. They observed a degradation of 90% in quercetin concentration, while miricetin and taxifolin decreased in 23% and 26%, respectively, and kaempferol was completely degraded. They concluded that degradation of these phenolic compounds may be due to the presence of free radicals generated during the plasma treatment with argon gas.

The energy of plasma produced free radicals (**Moisan *et al*., 2002; Abramzon *et al*., 2006; Kirkpatrick *et al*., 2007; Peña-Eguiluz, *et al*., 2010b**), and this caused changes the chemical structure of polyphenols, producing differences in total polyphenol content of treated chamomile and cinnamon samples (**Grzegorzewski *et al*., 2009**). Other studies in soy gamma radiation (1-10 kGy) showed an increase of 10% in polyphenols content and this increase may be due to polymerization of phenolic compounds and fragmentation of its chemical structure (**Štajner *et al*., 2007**). Furthermore, **Al-Nimer *et al*.**, (**2009**), conducted a studied on medicinal plants (cinnamon and green tea) treated with X-rays, resulting in a decrease of polyphenols content of 48.9% in cinnamon and 52% in green tea.

It is hard to explain the exact mechanism of decrease or increase in the polyphenol content in the studied samples of chamomile and cinnamon powder. We know from other studies that polyphenol content is linked to the antioxidant activity of these compounds and to free radicals production, depending on their molecular structure which undergoes changes, according to the method used as treatment for research the activity antioxidant (**Justino and Vieria, 2010; Floegel *et al*., 2011**).

Reduction of yeasts and molds in chamomile and cinnamon

Yeast and molds counts in chamomile samples before application of plasma treatment were 4.3±0.45 log CFU/g (Fig. 5). The reduction in the counts of molds and yeasts showed significant variations ($p<0.05$), at 650 V was 1.0±0.72 log CFU/g, while that 750 and 850 V there was not statistically significant difference and the final counts of yeasts and molds were <1.0 log CFU/g after 10 min of treatment.

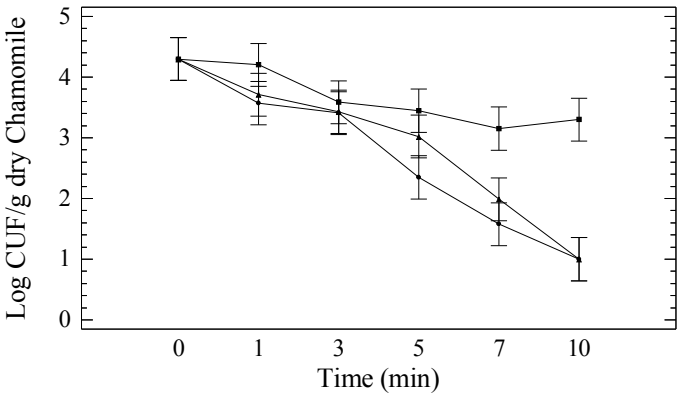

Figure 5 Yeast and molds counts (log CFU/g) in chamomile (*Matricaria chamomilla*) samples, treated with plasma at 650 (▪), 750 (▲) and 850 (●) Volts

In regard to yeasts and molds counts in cinnamon samples (Fig. 6), they showed an initial concentration of 3.8 ±0.13 log CFU/g. The reduction in the counts of molds and yeasts showed significant variations ($p<0.05$), between 650and 850 V with 0.17±0.21and 0.68±0.18 log CFU/g, while that 750 V the final counts of yeasts and molds was 0.58±0.18 log CFU/g after 10 min of treatment, this was homogeneous at 650 and 850 V.

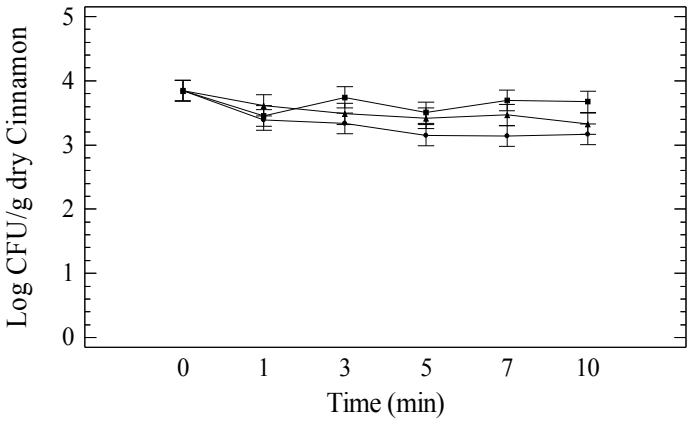

Figure 6 Yeasts and molds counts in cinnamon (*Cinnamomum zeylanicum*) samples treated with plasma at 650 (▪), 750 (▲) and 850 (●) Volts

The statistical results showed that there is no significant difference between treatments in the different times of 0-10 minutes, only between 850 V was different about that 650 and 750 V ($p<0.05$). The differences in the reduction of yeasts and molds counts observed in cinnamon samples after plasma treatment, may be influenced by sample density (0.69 g/cm3), providing a small interstitial space for plasma penetration, in comparison to chamomile sample that showed a lower density (0.19 g/cm3).

Decimal reduction times observed at three different voltages of plasma treatment are shown in Table 1. During inactivation of microorganisms, different types of survival curves could be observed, which will depend on the type of

microorganism and its survival ability in the substrate (**Laroussi, *et al*., 2003**).
We observed a survival curve with double slope, this "bi-phasic"

Table 1 Decimal reduction times (D-values) for yeasts and molds in chamomile (*Matricaria chamomilla*) and cinnamon (*Cinnamomum zeylanicum*) samples treated with plasma energy at different voltages.

	Chamomile (min)[1]		**Cinnamon** (min)[1]	
Voltage	D_1	D_2	D_1	D_2
650	7.69±4.60	32.67±21.21	12.23±3.11	93.57±27.26
750	6.03±4.45	3.13±1.98	12.39±9.10	28.30±21.20
850	3.10±1.08	4.26±1.94	13.87±14.68	297.62±84.18

[1] D_1 and D_2 values expressed in minutes correspond to the average of three replicates.

In chamomile, the decimal reduction D, presented two voltage values for each treatment, at 650V the value D1, showed a rapid decrease 7.69 ±4.60 $(min)^{-1}$, while the D2 was 21.21 ±32.67$(min)^{-1}$. However at 750 and 850 V, the values of D1 and D2 comprised between 3.6 and 3.10±4.45±1.08 $(min)^{-1}$. While cinnamon, the values of D1 and D2, realized values from 12.23 ±3.11 to 297.62 ±84.18 $(min)^{-1}$, still below the values obtained for the samples of chamomile.

Referent this values, the probably indicates that during the first phase of plasma treatment, the cell surface suffers damage, leading to pore formation. According to **Moisan *et al.* (2002)**, in the second phase, we assuming that free radicals or reactive compound formed during exposure to plasma energy rapidly penetrate into the cell due to the pores in the cell surface, accelerating cell death. They studied the effect of different physicochemical processes during plasma sterilization, based on specific characteristics of *B. subtilis* spores survival curves. They interpreted of survival curves and assumed from the inactivation kinetics three probable actions of plasma on the spores: 1) microbial DNA injury or destruction by UV radiation: 2) Formation of photon induced desorption results from UV photons, breaking chemical bonds leading to the formation of pores in the membrane, caused by volatile compounds formed from atoms of the microorganism; and 3) Erosion of the microorganism through etching stems from the adsorption of reactive species from the plasma (glow or afterglow) on the microorganism with which they subsequently undergo chemical reactions to form volatile compounds (spontaneous etching). The reactive species can be atomic and molecular radicals, for example, O and O_3, respectively, and excited molecules in a metastable state, for example, the O_2 singlet state (**Justino and Vieria, 2010**). We believe that, in all cases, the inactivation of yeasts and molds may be the result of DNA destruction by UV photons produced by plasma energy.

CONCLUSION

Exposure of chamomile and cinnamon samples to plasma energy reduced <1.0 log CFU/g at 750 and 850 for chamomile and 0.68±0.18 log CFU/g for cinnamon at 850 V after 10 min of treatment respectively. The antioxidant activity of the total phenolic compounds contained in chamomile and cinnamon was affected by application of plasma energy. The treatment reduced the antioxidant activity (IC_{50}) up to 55% at 850 V in chamomile after 10 min of treatment. In general, the application of plasma energy may be an alternative to reduce fungal growth in dried foods. We conclude that the best treatment to obtain significant reductions on yeasts and molds counts in chamomile and cinnamon samples with the lowest degradation of antioxidant compounds was the application of plasma energy at 750 V for 10 min.

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