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*Research Article*

# Effect of the Application of Cold Plasma Energy on the Inactivation of Microorganisms, Proteins, and Lipids Deterioration in Adobera Cheese

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Cheeses are perishable foods that must fulfill sanitary and quality requirements according to the parameters established globally. Plasma as a nonthermal inactivation technique has been a current research topic for food preservation, so the objective of this work was to study the effect of plasma energy against microorganisms in Adobera cheese (traditional Mexican cheese) as well as evaluate the possible degradation of lipids and protein. 108 CFU/mL of *Escherichia coli* ATCC 25922, *Salmonella* ATCC13076, and *Staphylococcus aureus* ATCC 6538 were inoculated at 0.5 g of Adobera cheese and were subjected to an energy of 30 volts, in a dielectric barrier discharge reactor (DBDR) at intervals of times 1, 3, 5, 7, 10, and 15 min. A flow of a mixture of air and helium at 96% purity was used. The decimal reduction time (D) was determined, and the oxidation of proteins and lipids was analyzed after each treatment. The results showed an annihilating effect of plasma on the indicator bacteria under study, and a reduction of 5 logarithmic cycles was obtained. The maximum degree of lipid oxidation was 23 acid degree values (ADV) after 7 min of exposure to plasma. The oxidation of proteins showed a direct and proportional relationship between the formation of carbonyl groups with the percentage significant loss to the concentration of carbonyl groups with the concentration of protein oxidation, after 3 min of exposure to cold plasma levels of 82% and 99% oxidation of Adobera cheese protein and free casein, respectively. We conclude that the plasma energy applied to Adobera cheese is an effective treatment to inactivate bacteria. However, there is the possibility of causing changes in taste and odor, due to the release of fatty acids and the oxidation of proteins.

## 1. Introduction

Adobera cheese is a traditional Mexican cheese, originated in the Jalisco state, made from nonpasteurized cow's milk that does not follow a maturing process. This kind of cheese has a firm, crumbly, and grainy texture, and it is soft and salty flavored. The name Adobera comes from the shape of this cheese, which is similar to an adobe brick. This cheese could be added with chilli or spices depending on the manufacturing region [1, 2]. Adobera cheese is the most commonly consumed artisanal Mexican cheese in Jalisco and shows potential for a protected designation of origin. Due to the scarcity of scientific studies, there is a need of developing technological strategies to decrease pathogenic microorganisms during cheese manufacture, storage, and distribution [3].

Cold plasma (CP) energy is an emerging technology, which appears today as an interesting option for the food industry, mainly to satisfy the consumer demand for fresh products, in addition to its ability to maintain low temperatures during operations, reduce the use of toxic gases that impact health and environmental safety [4]. The CP is generated under atmospheric or vacuum conditions at room temperature and requires little energy. Simply, the process of plasma formation can be described as the neutral gas being ionized with low energy. The CP is a fully ionized gas mixture of various reactive species such as UV photons, charged particles, free electrons, positive and negative ions, free radicals, excited state atoms, and reactive oxygen species (ROS), and nitrogen such as superoxide ($O^{2-}$), hydroxyl radical, singlet oxygen, atomic oxygen, hydrogen peroxide, ozone ($O_3$), nitrogen dioxide ($NO_2$), and nitric oxide (NO) [5–7]. The antimicrobial potential of CP, together with the species molecular oxygen ($O_2$), superoxide anion ($O_2^-$), ozone ($O_3$), hydrogen peroxide ($H_2O_2$), hydroxyl ($OH^-$), peroxyl ($ROO^-$), hydroperoxides (ROOH) among others has been thoroughly studied and described by various authors [5, 7–10].

Some studies have shown the application of CP in wounds to eliminate methicillin-resistant strains of *S. aureus* and of biofilms formed by multiresistant bacteria [8, 11–13]. On the other hand, Fernandez and Thompson (2012), evaluated the CP against *Escherichia coli*, *Saccharomyces cerevisiae*, *Gluconobacter liquefaciens*, and *Listeria monocytogenes* in slices inoculated on mango (*Mangifera indica*) and cantaloupe melon (*Cucumis melo* var. reticulatus) surface, observing that it was very efficient in reducing the microbial load on the surfaces of these fruits [9].

The discharges of plasma, within both a parallel plate and a coaxial dielectric barrier discharge reactor (DBDR), at atmospheric pressure with a He-air gas mixture at a 1.5 L/min flow, have been successfully applied to bacteria removal in short periods of time processing [14–18]. Nonthermal plasma has been also applied to inactivate *Staphylococcus aureus* and *Saccharomyces cerevisiae*, exposing the cells under a flow of 1.5 L/min of a He-air gas mixture and 850 V, generated by nonthermal plasma with a DBDR. The cells of *S. aureus* showed membrane damage while *S. cerevisiae* had damage to its cell wall, with consequent DNA denaturation [19]. Regardless of the effects of cold plasma on microbial elimination, there are other studies that have evaluated the effects of cold plasma energy in relation to antioxidants, proteins, and fats [20–23]. Regarding the antioxidant activity of some polyphenols contained in cinnamon and chamomile, it was observed in this study that cold plasma energy can cause a decrease in antioxidant activity depending on the origin of some polyphenols [20]. On the other hand, the energy of the cold plasma causes changes in the structure of the peptide chain in proteins, altering biological properties, such is the case of bovine serum albumin and soybean agglutinin [21, 22]. Another study shows alterations that occur in lipids, generally their oxidation such is the case of a study carried out on chicken meat [23].

Minimal changes in the physical, chemical, nutritional, and sensory attributes of various products have been obtained using CP treatments due to their nonthermal nature. Then, this technology can be effective as an alternative tool for food decontamination and shelf-life extension. Moreover, the limited studies on the interactions of species reactive, with food components at the molecular and nutritional level offer big research opportunities [5, 18].

Since the interest in the application of CP in food for its preservation has grown, this work aimed to study the inhibitory effect of CP in Adobera cheese inoculated with *Escherichia coli* ATCC 25922, *Salmonella* ATCC13076, and *Staphylococcus aureus* ATCC 6538, as well as to evaluate the possible degradation of lipids and protein.

## 2. Materials and Methods

*2.1. Biological Material.* For this study, we used Adobera cheese slices with a thickness of 0.2 cm and an approximate weight of 0.5 g. Individual cheese slices were inoculated with 50 $\mu$L of each bacterium (*Escherichia coli* ATCC 25922, *Salmonella* ATCC13076, and *Staphylococcus aureus* ATCC 6538) just like, mixing the three strains, with a concentration of 0.5 on the McFarland scale, equivalent to 108 CFU/mL.

*2.2. Treatment of Adobera Cheese with Dielectric Barrier Discharge Reactor (DBDR).* The cheese slices inoculated with the bacteria were exposed to non thermal plasma generated by a DBDR designed and built by the National Institute for Nuclear Research (ININ, Mexico) [19]. This device operates at 13.56 MHz and is driven by a specifically built radio frequency (RF) resonant converter. The reactor, which operates at atmospheric pressure in a He-air gas mixture, with 96% purity, and 1.5 L/min flow rate, at 30 W input power and an output voltage of 850 V. The cheese samples will have cold plasma exposure treatment at different exposure times (1, 3, 5, 7, 10, and 15 min). The microbiological analysis of the cheese slices was performed after plasma exposure.

*2.3. Microbiological Analysis.* The microbiological analysis was carried out by introducing the Adobera cheese slices after CP treatment, in a test tube containing 6 mL of physiological saline solution; the tube was shaken in a vortex allowing the cheese particles to precipitate. Then, 1 mL of the supernatant was taken and placed inside Petri dishes with selective agar according to each strain (Mac Conkey Salt and Mannitol agar and Salmonella Shigella agar, BD Bioxon™, México), the Petri dishes were then incubated at 37°C for 24 and 48 hours [24].

*2.4. Decimal Reduction (D) Kinetics.* Decimal reduction (D), which is the time required to inactivate 90% of the microbial population on a logarithmic scale, was calculated using the kinetics of death obtained from the treatment of cheese samples at different times of exposition to plasma energy [25, 26]. This parameter was obtained by calculating the slope of the kinetic equation as follows.

$$\log\frac{N}{N_0} = \log\left(e^{-kdt}\right),$$

$$\log\frac{N}{N_0} = -\frac{kdt}{2.3}, \quad (1)$$

$$D = \frac{2.3}{Kd},$$

where $N$ is the number of initial microorganisms, $N_0$ is the number of final microorganisms, $Kd$ is the kinetic constant of death, and $t$ is the time.

*2.5. Protein Oxidation.* After exposure of Adobera cheese to plasma energy, the cheese slices were suspended in 0.9% saline solution and vortexed until the samples were homogenized. 10 $\mu$L of the supernatant was taken and transferred to an Eppendorf tube containing 500 $\mu$L of 0.2% 2,4-dinitrophenylhydrazine DNFH (w/v) in 2N HCl. The mixture was incubated for 1 h at room temperature. Subsequently, 500 $\mu$L of 20% (w/v) TCA was added. The sample was incubated for 15 min at 4°C to promote protein precipitation. The tubes were centrifuged at 6000 g for 3 min in a refrigerated centrifuge (Biosan, LMC-4200R, USA), discarding the supernatant. The precipitate was washed three times with 1 mL of a mixture of ethanol: ethyl acetate (1 : 1), centrifuging each time and removing the supernatant. The precipitate was dissolved in 1 mL of guanidine (6 M in 20 mM potassium phosphate), vortexed, and incubated at 37°C for 30 min. Finally, the samples were analyzed by using a UV/vis spectrophotometer (Jenway 6350316305, United Kingdom) at 360 nm, with a guanidine blank. The same procedure was performed using 1g of casein (Hy-Case® Amino, Merck, USA). The results were expressed as nanomoles of free carbonyl groups/mg of total protein [27].

*2.6. Hydrolytic Rancidity.* Samples of 1 g of Adobera cheese were subjected to plasma energy treatment at different times and were transferred to a 50 mL tube. After, 2 mL nonionic surfactant reagent (30 g of Triton X-100 and 70 g of sodium sulfate dissolved in 1L of distilled water) was added and vortexed until homogenized. The tubes were placed in a bath with a hot water shaker at 57°C for 20 min. Subsequently, 2 mL of methyl alcohol was added to each tube, 200 $\mu$L of fat were extracted and transferred into a tube and dissolved in 1 mL of solvent for fat (petroleum ether: n-propanol ratio 4 : 1 v/v and methyl alcohol: water 50 : 50). Finally, 2 drops of phenolphthalein were added and the samples were subsequently titrated with KOH 0.01 N. Lipid oxidation was measured as the degree of free fatty acid (ADV), defined as milliequivalents of alkali (KOH) per 100 g of fat and calculated as follows [28]:

$$\text{ADV} = \frac{((\text{mL KOH} - \text{mL of KOH for blank}) \times N \times 100)}{\text{Weight of fat of sample}}, \quad (2)$$

where $N$ is the normality of KOH solution in the volume of titration (with sample and blank), the density is 1.033 g/cm$^3$, and 14% total fat in cheese.

*2.7. Statistical Analysis.* All analyses were performed in triplicate and treatments were realized three times. The data were reported as mean ± standard deviation (SD) or standard error of the mean (SE). Statistical analysis was performed using the STATGRAPHICS Centurion XV software version 2.15.06. Analysis of variance (ANOVA) and multiple comparison procedures (least significant difference, LSD) tests were conducted to determine whether there were significant differences ($p < 0.05$) among treatments.

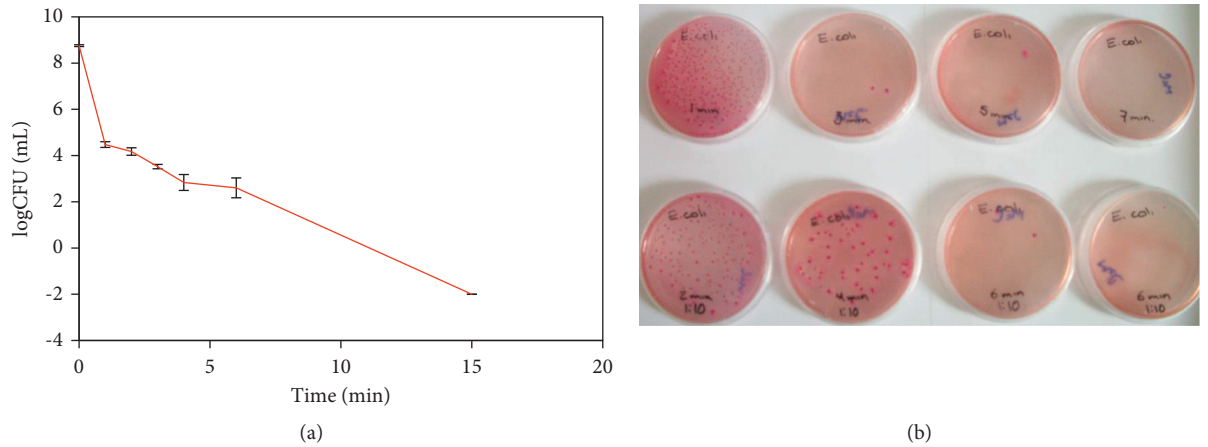
## 3. Results

*3.1. Microbiological Analysis of Adobera Cheese.* After 15 min of treatment with CP, the population of *Escherichia coli* ATCC 25922, *Salmonella* ATCC13076, and *Staphylococcus aureus* ATCC 6538 was reduced 5 logarithmic cycles. For the whole strain, two different phases of death time were observed during death kinetics (Figures 1(a), 2(a), and 3(a)). The first phase showed an accelerated death of the bacteria, managing to reduce the cell viability by 5 logarithms. Then, a resistance of the bacteria to die in the second phase was observed. However, it can be observed in the Petri dish cultures that after 7 minutes of CP treatment, there is no growth of any of the three bacteria (Figures 1(b), 2(b), and 3(b)).

Regarding the parameters of the microbial death constant (*kd*) and microbial decimal reduction (D) (see Table 1), we can observe that the values are very similar between the three strains individually (*Escherichia coli* ATCC 25922, *Salmonella* ATCC13076, and *Staphylococcus aureus* ATCC 6538) and do not show significant differences ($p > 0.05$). On the contrary, when comparing the treatment with the mixture of the three indicator strains, differences in both *Kd* and D are presented in both slopes $p < 0.05$. In previous studies, the cells of *Escherichia coli*, *Staphylococcus aureus*, and *Saccharomyces cerevisiae*, showed membrane damage with pore formation, caused by the action of nonthermal plasma, which leads breakdown on membrane with consecutive DNA denaturation, these being the main factors inducing death or inactivation of the microorganism [19].

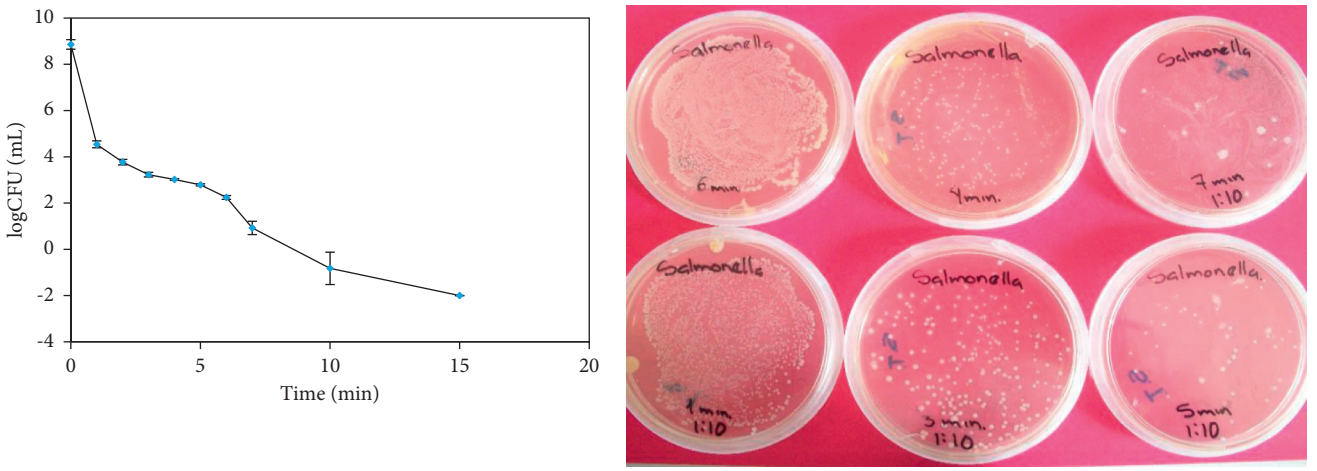
Regarding the results obtained from the treatment with CP in the mixture of 3 bacteria (Figure 4(a)), this test presents death kinetics very similar to that observed for *Escherichia coli* ATCC 25922, *Salmonella* ATCC13076, and *Staphylococcus aureus* ATCC 6538, each one separately. Similarly, a 5-log reduction in death was observed, and 2 phases of death were registered (Figures 1, 2, and 3). Similar cell reductions were observed in the mixture of bacteria treated with CP during different times (Figures 4(a) and 4(b)).

*3.2. Proteins and Hydrolytic Rancidity.* The proteins are susceptible to oxidative reactions that lead to deterioration of their quality since carbonylation is an irreversible



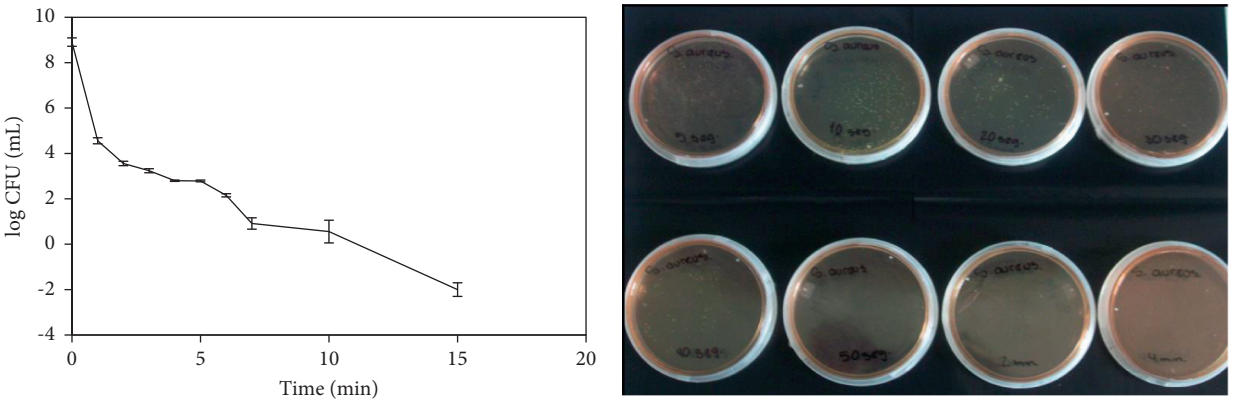

(a) (b)

Figure 1: Biomass reduction. (a) Death kinetics of *Escherichia coli* ATCC 25922; (b) *Escherichia coli* ATCC 25922 cell in Petri dishes.




(a) (b)

Figure 2: Biomass reduction. (a) Death kinetics of *Salmonella* ATCC13076; (b) *Salmonella* ATCC13076 cell in Petri dishes.




(a) (b)

Figure 3: Biomass reduction. (a) Death kinetics of *Staphylococcus aureus* ATCC 6538; (b) *Staphylococcus aureus* ATCC 6538 cells in Petri dishes.

Table 1: Mean death constant (± SD)* and time of death in seconds (± SD)* of the different bacterial strains, in slopes 1 and 2.

| Strains | Pending 1 | | Pending 2 | |
|---|---|---|---|---|
| Died time | *Kd* | D | *Kd* | D |
| *E. coli* | 9.84 ± 0.34 | 0.23 ± 0.008 | 0.87 ± 0.18 | 2.71 ± 0.56 |
| *Salmonella* | 9.93 ± 0.71 | 0.23 ± 0.01 | 1.35 ± 0.31 | 1.78 ± 0.44 |
| *S. aureus* | 9.93 ± 0.71 | 0.23 ± 0.01 | 1.22 ± 0.47 | 2.17 ± 0.98 |
| Bacteria mix | 11.32 ± 0.10 $p < 0.05$ | 0.2 ± 0.001** | 0.35 ± 0.05** | 6.60 ± 0.98** |

*SD: standard deviation; **Statistically significant difference with each individual bacteria, ANOVA; $p < 0.05$, *Kd*: death constant, D: death time in minutes.

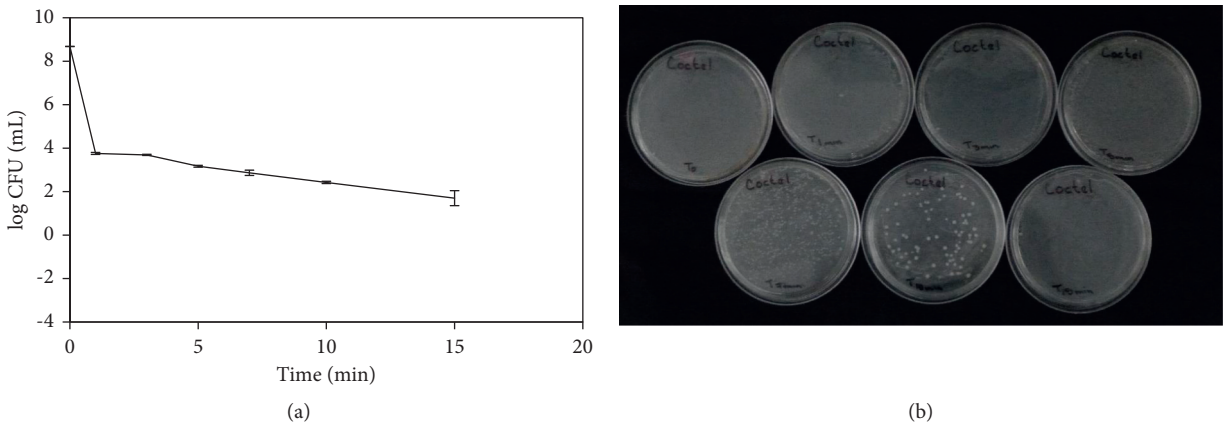



Figure 4: Biomass reduction. (a) Death kinetics of the mixture of bacteria; (b) Mixture of bacteria cells in Petri dishes.

modification that forms carbonyl groups induced by oxidative stress and other mechanisms.

The Adobera cheese contains approximately 4.2 g of total fat and 6.1 g of total protein per kg. The amounts of protein-bound carbonyl functional groups were used as markers to evaluate the oxidative damage during CP treatment (Figure 5). Before plasma treatment, the concentrations of carbonyl functional groups in the cheese were practically zero, but once the samples were subjected to the CP treatment, the concentrations were altered. After 1 min of exposure to the CP, an increase of carbonyl functional groups was observed, nevertheless, no significant difference ($p > 0.05$) was observed among treatments at 3 to 15 min. The total percentage of carbonyl oxidation was 99% and a maximum of carbonyl groups of 1.62 nmol/mg of protein (Figure 5(a)).

We also verify if the CP will produce the same oxidative damage on free casein (Figure 5(b)). The results showed significant differences between the CP treatments of 0 and 1 at 3 min ($p < 0.05$) in casein, a maximum of carbonyl groups of 10.89 nmol/mg protein was produced at 3 min, this corresponds to 79% of oxidized protein. No significant differences were observed among 3, 15, and 25 min ($p > 0.25$), exhibiting behavior of oxidation very similar to that in Adobera cheese proteins.

A significant difference was observed in the concentration of carbonyl groups between Adobera cheese and casein, being 1.62 and 10.89 nmol/g of the sample, respectively. We assume that free casein, when subjected to CP directly, exposes some amino acids more directly, and these are altered in carbonyl groups, while Adobera cheese is a complex matrix of multicomponent with high water content (55%) where the exposure of its amino acids is not as high as casein.

On the other hand, it is well known that when foods with a high-fat content come into contact with light and/or temperature, the fat is oxidized and the sensory characteristics of the product are modified. The oxidation of lipids in cheese (Figure 6) shows ADV values of 12, 19, 20, and 21 corresponding to the times of 1, 3, 5, and 7 min of treatments, respectively. The Standard Method for the Examination of Dairy Products of the American Public Health Association (1972) dictates that a value of ADV < 0.4 is acceptable oxidation in the product, a value of 0.7 to 1.1 is at the limit of its acceptance, a value of 1.2 shows slight rancidity and a value of 1.5 shows extreme hydrolytic rancidity, generating a very noticeable change in flavor and texture.

## 4. Discussion

Cold plasma (CP) is an emerging technology, which has been used as an alternative method for sanitization in the food industry because healthy foods with a fresh-like appearance are nowadays demanded by consumers [29]. It has been a study that reactive oxygen and nitrogen species (ROS and RNS) generated by cold atmospheric pressure plasma damage DNA of microorganisms. ROS and RNS generated by plasma could induce DNA-protein crosslinks (DPCs) in bacteria, yeast, and even human cells. Some examples of highly oxidized free radicals within the CP cloud are $O_2$ molecular oxygen ($O_2$), superoxide anion ($O_2^-$), ozone ($O_3$), hydrogen peroxide ($H_2O_2$), hydroxyl ($OH^-$), peroxyl ($ROO^-$), and hydroperoxides (ROOH) among others [30].

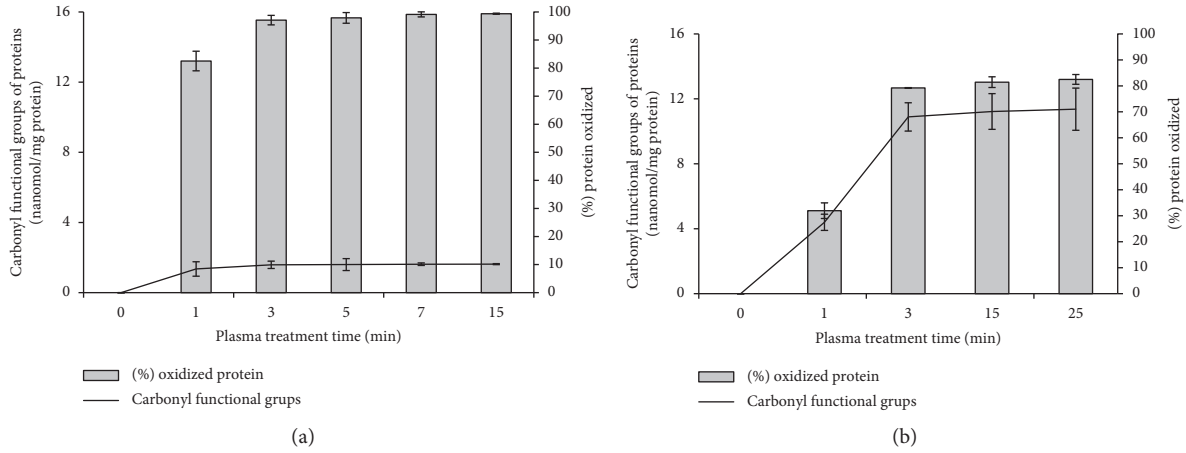



Figure 5: Protein oxidation damage after CP treatment. (a) Treatment with CP of Adobera cheese. (b) Treatment with CP in free casein.

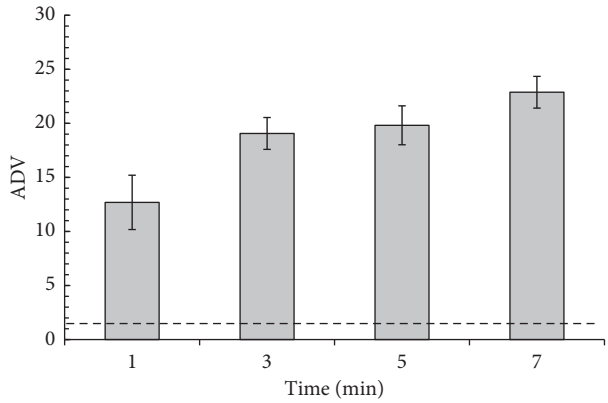



Figure 6: Hydrolytic rancidity (AVD) in Adobera cheese after CP treatment. The dotted line represents the limit value of ADV, a value > 1.5 ADV represents high rancidity.

We obtained similar results to the study conducted by Dezest et al. [31], where three different types of plasma or gas mixtures (helium alone or with 1% oxygen or 1% nitrogen) were used against *E. coli*. The result obtained with He-$O_2$ plasma was the most aggressive against *E. coli* and showed faster bactericidal effects. Oxidative stress caused by plasma treatment leads to significant damage to bacteria, especially membrane leakage and morphological changes. Biochemical analyses of *E. coli* macromolecules indicated massive intracellular protein oxidation. However, reactive oxygen and nitrogen species which are not the only actors involved in *E. coli* death, electric field, and charged particles could play a significant role. In other studies, they used dielectric barrier discharge (DBD) and surface barrier discharge (SBD) to analyze their efficacy against *Salmonella typhimurium* biofilms and *Listeria monocytogenes* strain, at different plasma intensities (13.88, 17.88, 21.88 V input voltage). They obtained a reduction up 3.5 log10 in both bacteria, using the DBD electrode, 0.0 (v/v)% $O_2$, and an input voltage of 21.88 V [32].

In our work, the CP treatment was efficient in inactivating pathogenic bacteria inoculated on Adobera cheese. The values of D obtained from two resulting slopes, in the case of the mixture of bacteria, were 0.20 and 6.60 minutes, respectively, decreasing 6 logarithmic cycles. A study similar to ours [33] shows the efficacy of atmospheric pressure plasma (APP) in sliced cheese and ham inoculated with a 3-strain cocktail of *Listeria monocytogenes*. The process parameters were input power (75, 100, 125, and 150 W) and plasma exposure time (60, 90, and 120 seconds). After 120 seconds of APP treatment at 150 W, which presented greater intensity of the plasma cloud and ability to eliminate cases. The viable cells of *L. monocytogenes* were reduced by 8 log reduction, while the reductions of *L. monocytogenes* in sliced ham, after 120 seconds *s* ranged from 0.25 to 1.73 log CFU/g. The D value obtained to 150 W was 17.27 seconds for in-sliced cheese and in-sliced ham was 63.69 seconds. These results indicate that the inactivation effects of CP treatment on *L. monocytogenes* studied by Song et al., 2009 [33], compared with the mixture of bacteria (*E. coli* ATCC 25922, *Salmonella* ATCC13076, and *Staphylococcus aureus* ATCC 6538) inoculated on Adobera cheese, are strongly dependent on the type of food to be treated with cold plasma.

Wan et al. (2019) demonstrates through a surface analysis of scanning electron microscopy, in fresh cheese inoculated with *Listeria innocua*, that the differences in the roughness of the surface and the microstructure of cheese, have a great impact on the efficacy of the cold plasma treatment, since the roughness or surface protects microorganisms preventing effective penetration of CP [34].

In our case, with the cold plasma treatment, graphs were obtained with a system of different slopes, obtaining two different decimal reductions. The first D in all treatments of the treated bacteria causes severe damage in a short time. While the second D corresponds to the change in slope with a value greater than the first, indicating what damage it will generate will take longer but following the continuity of the damage (see Table 1). Moisan et al. citing that the presence of several slopes indicates the microbial reduction is due firstly to destruction by UV irradiation of the genetic material of the microorganism. A second phenomenon is caused by an erosion of the microorganism, atom by atom, through intrinsic photodesorption and finally another erosion of the microorganism, atom by atom, resulting from the

adsorption of highly oxidizing reactive plasma species in the microorganism with which they subsequently undergo reactions with chemicals to form volatile compounds [35]. Another study shows how treatment with CP destroys the cell wall, releasing microbial cytoplasmic material and with the help of propidium iodide, which binds to DNA, causing fluorescence that can be observed under a fluorescence microscope. This study also corroborated how the microbial DNA was degraded by extracting and electrophoresis of the DNA obtained at different treatment times [19].

The effect of CP for reducing microbial load depends on several factors, the most important are the voltage and frequency, input power, treatment time, type and composition of the gas, and flow rate and exposure mode of the sample to be sterilized. Another factor is the reactive species and free radicals produced by plasma, that can interact with phospholipids and proteins present in the cell membrane causing ionic and chemical interactions, thus destroying the plasma membrane [29].

In theory, proteolysis and hydrolytic rancidity have been considered as one of the main causes of loss of nutritional quality, as well as, a cause of concern for food safety in the cheese [36]. In Adobera cheese, some changes in texture, such as softening and color change were observed; then, the oxidation of proteins and fat could be the cause of these changes. Since flavor compounds are more soluble in fat than in water, lipid oxidation could cause the flavor to lose. The proportion and structure of fat may affect the rheological properties of the cheese such as the texture [37]. The results obtained in Adobera cheese and casein samples undergoing CP treatment were similar regarding the behavior of the oxidation curve of the carbonyl groups.

However, Adobera cheese presented a high percentage of protein oxidation, probably due to the amino acids with functional groups capable of being directly oxidized in side chains such as lysine, threonine, arginine, and proline [38].

Prolonged treatment of CP oxidizes proteins, altering amino acids and secondary, tertiary, and quaternary structures of the same causing conformational changes and unfolding of proteins leading to fragmentation, formation of crosslinks, unfolding and conformational changes. Arginine, histidine, lysine, proline, threonine, and tryptophan undergo a carbonylation reaction as a result of ROS attacks managed by CP [39]. On the other hand, a protein isolated from serum was treated with CP, and it causes a modification in the groups of the side chain of some amino acids, especially with $NH^{-}$ or $NH_2$, the division of the peptide bonds and the increase of carbonyl functional groups [39].

In our work, hydrolysis or protein structure changes tests were not performed, but there are reports of protein denaturation and aggregate formation have been reported after exposure to cold plasma-treated chocolate milk drinks causing undesirable flavors and odors [40]. On the other hand, another study indicates that the cold plasma effect may not cause significant changes on sensory properties of dairy products, if low pressure (16 Pa) or nitrogen gas is applied, since there is no formation of ROS. However, it may result in mild lipid oxidation or significant protein aggregation if using air and high voltage (60 kV), with prolonged treatment time of more than 30 minutes, causing in product quality losses and off-flavor [41].

Lipolysis is the hydrolysis of triglycerides normally produced by enzymatic activity. As a result of lipolysis, free fatty acids are produced together with other products in later stages of degradation, such as alcohols, esters, aldehydes, ketones, and lactones. These are the main organic volatile compounds of the characteristic aroma and flavor of cheeses [42]. The ROS mainly generated in the cold plasma cloud interacts with lipids breaking the double bonds of unsaturated fatty acids and forms an acid radical, where it would then be oxidized into lipid hydroperoxides. Hydroperoxides can initiate different reactions such as the decomposition of lipids or the union of fatty acid chains through the formation of ether or peroxide bridges. Hydroperoxides are converted to shorter chain fatty acyl compounds and aldehydes. Secondary oxidation products, such as aldehydes, cause other reactions in food, generating unpleasant flavors and negatively affecting the sensory quality of the product [39].

In our study, as a consequence of the CP treatment in Adobera cheese, changes in some organoleptic characteristics were observed due to the oxidation of proteins and lipids contained in the cheese. There is an urgent need to regulate food safety through effective CP sanitation without compromising food quality. The application of atmospheric CP is investigated for its food application, mainly as a tool for the elimination of microorganisms as a disinfection step. This has led to an increased interest in the use of CP to disinfect meat, poultry, and dairy products, so it is necessary to continue with more studies to evaluate the efficacy of cold plasma in the processing of different food products [43].

## 5. Conclusions

The dielectric rod discharge reactor is an effective method for the inactivation of microorganisms in sliced Adobera cheese. It was possible to reduce the cell concentration of each strain for more than 5 logarithmic cycles, as well as, in a mixture of 3 indicating bacteria. On the other hand, the treatment applied to Adobera cheese caused severe oxidation, both of proteins and lipids, causing alterations in texture and smell. It is important to carry out further studies to optimize the time, flow, and energy parameters of the cold plasma for an application where a balance point can be found between the reduction of the microbial population and the oxidation of proteins and lipids. We demonstrate both the application and the limitations of CP treatments in Adobera cheese, therefore, we recommend that in future cold plasma research in dairy products should consider not only microbial inactivation as part of safety and quality of the food but also to verify the nutritional quality and the sensory perception of the consumer.

## Data Availability

The data used to support the findings of this study are included within the article.

## Conflicts of Interest

The authors declare no conflicts of interest.

## Authors' Contributions

A.U and S.P conceptualized the study, provided the resources, and administered the project. A.U, S.P, and C.S designed the methodology. A.U, S.P, R.S, and A.D performed the formal analysis. A.U, S.P, P.M, and C.S performed the investigation. B.L, R.S, and C.S curated the data. A.U, J.S, A.D, and R.S wrote the original draft. R.S, C.S, P.M, and B.L wrote the review and edited the manuscript. All the authors have read and agreed to the published version of the manuscript.

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