Iranian Journal of Microbiology
Volume 16 Number 1 (February 2024) 62-67

ORIGINAL ARTICLE

Investigating the effect of cold atmospheric plasma treatment on the microbial load of raw potato slices

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Received: August 2023**, Accepted:** January 2024

ABSTRACT

Background and Objectives: Potatoes (*Solanum tuberosum*), as starchy plants, have been highly esteemed for their rich supply of nutrients. Numerous studies have investigated the potential health benefits of potatoes and explored potential solutions. Among these considerations, the discussion regarding microbial contaminants has remained an important topic.
Materials and Methods: The present study used cold atmospheric plasma (CAP) to evaluate the microbial quality (including mesophilic and psychrotrophic bacteria and mold and yeasts) of raw potato slices during a 14-day storage period. To achieve this goal, the duration of CAP exposure was set at 5, 10, and 15 min, utilizing an electric voltage of 60 kV and a specific frequency of 20 kHz.
Results: The findings revealed the effectiveness of CAP pre-treatment in inhibiting microbial growth over the 14 days when compared to the control sample (untreated sample), with a statistically significant difference ($P < 0.05$). Moreover, with an extension of the CAP exposure duration to 15 min, there was a significant reduction in the logarithmic count of mesophilic, psychrotrophic microorganisms, molds, and yeasts (4.95, 2.85, and 2.22CFU/g, respectively) in comparison to the control groups (7.5, 5.62, and 5.5CFU/g) on days 0, 7, and 14 of the storage periods ($P < 0.05$).
Conclusion: The results of this study highlight the potential of CAP pre-treatment on reducing the microbial load in raw potato slices prior to frying, which could potentially influence the overall quality of potato-based products.

Keywords: Bacteria; Cold plasma; Fungi; Storage; *Solanum tuberosum*

INTRODUCTION

Potatoes have recently been increasingly recognized as a valuable and nutritious food source (1). They are typically categorized as "starchy vegetables" given their abundant carbohydrate content (2). Starch is the primary carbohydrate in potatoes, consisting of two main components; amylopectin and amylose (3). Potatoes contain a small portion of starch that is "resistant" to enzymatic breakdown in the small intestine, allowing it to reach the large intestine intact. This "resistant starch" undergoes extensive fermentation by the colonic microflora, producing short-chain fatty acids. These fatty acids play

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roles in lowering intestinal pH, reducing toxic levels of ammonia in the digestive tract, and acting as a prebiotic by promoting the growth of beneficial bacteria in the colon (2, 4).

Newly, there has been a notable increase in the demand for safe foods with excellent nutritional quality (5-7). Hence, novel technologies, such as low-temperature approaches, have been introduced to preserve food while minimizing their effects on sensory attributes and nutritional content (7, 8). Cold plasma is a cutting-edge non-thermal technology that exhibits remarkable potential in deactivating various microorganisms, including bacteria, fungi, viruses, and spores (9, 10). Researchers generate cold plasma by applying electric or microwave energy to gases like air, oxygen, hydrogen, nitrogen, helium, argon, or combinations. This process is conducted at low pressure or atmospheric conditions (11-13). Using these gases and energy sources can create and control cold plasma for a wide range of applications, including sterilization, surface modification, food processing, and medical treatments (12-14). Cold plasma primarily achieves the inactivation of microorganisms through the disruption of chemical bonds within the microbial cells. This action causes damage to the cell membranes, leading to the release of macromolecules from the cells and ultimately resulting in cell death (15). The use of cold plasma technology attracted considerable attention in the context of food safety, owing to its advantages, such as low temperature and energy requirements, short processing time, and the absence of chemical residues on treated product surfaces (12, 16).

The antimicrobial efficacy of cold plasma technology has been extensively studied across various food products (10, 17, 18). Researchers have reported efforts to confirm the inactivation of virus and microbial cells by cold plasma (9, 18-20).

Microbial diseases are the most critical constraints of potato production, particularly in equatorial and subtropical regions and some warm temperate areas (21-23). Bacterial diseases have been reported to impact potatoes globally, causing significant damage, particularly to the tubers, which are economically the most crucial parts of the plant (22). Bacterial wilt and blackleg are among the most frequently reported diseases in potatoes and tomatoes (22, 24). Additionally, other diseases such as potato pink eye, ring rot, and common scab are also prevalent and can affect potatoes at various stages of their growth, including the juvenile (22, 25). For instance, the total significance of Egyptian potato exports has been negatively affected by the EU's quarantine restrictions on potato bacterial wilt also known as brown rot (26, 27). A survey was conducted in the Nile Delta of Egypt to assess the prevalence of potato brown rot. This severe and endemic disease is caused by *Ralstonia solanacearum* race three biovar 2 (25, 26). Given the significant annual losses experienced by the potato industry due to brown rot disease (25-27), the focus was on eradicating and managing the pathogen in potentially infected sites, as well as implementing brown rot control measures in infested areas. Potato bacterial wilt is caused by a bacterium that gains entry into the potato plant through various means, including natural openings, mechanical wounds, cracks, or rot tips. Once inside the plant, the bacterium colonizes the cortex, which is the sub-epidermal tissue of the plant. From there, it proceeds to infect the xylem vessels, which play a crucial role in conducting water and nutrients throughout the plant. As the bacterium spreads within the xylem, it disrupts the plant's ability to take up water. This blockage leads to wilting of the plant and, ultimately, the death of the potato plant (21, 25-27). In this regard, the impact of cold plasma seed treatment on tomato bacterial wilt was examined and found to significantly enhance tomato growth while effectively controlling tomato bacterial wilt (23). Given that potatoes are a staple ingredient globally, ensuring the quality of processed potato-based products by reducing microbial load before production holds great importance. Therefore, in the present study, the impact of CAP pre-treatment at specific voltage (60 kV) and various exposure times (5, 10 and 15 min) was investigated on microbial populations (precisely mesophilic and psychrotrophic bacteria as well as mold and yeasts) of raw potato slices.

MATERIALS AND METHODS

Material. Potato samples were collected randomly from the market in Tehran province, Iran. Potato dextrose agar (PDA), Plate Count Agar (PCA), and peptone water were obtained from Merck Co (Germany).

Potato slice preparation. Initially, the potatoes were washed, peeled, and sliced into pieces of 2 mm thickness and 22 mm diameter. The potato slices were then

rinsed with distilled water for approximately 1 min to remove any residues. Excess water on the potato surfaces was dried using a tissue, and the slices were left at room temperature (~30 min) for further analysis.

Dielectric barrier discharge cold plasma pretreatment. CAP was generated using a dielectric barrier discharge (DBD) system (Kavosh Yaran Fan Pouya Co, Iran) was employed as a pretreatment on potato slices under atmospheric pressure and ambient temperature with argon gas as the carrier gas. The plasma radiation duration was set at 5, 10, and 15 min, with an electric voltage of 60 kV, which was supplied through the use of a step-up transformer. Following this, both control samples (untreated with CAP) and pre-treated samples went through blanching by immersing the potato slices in boiling water for 3 min (sample to water ratio approximately 1:8). Subsequently, the blanched samples were cooled to room temperature, and surface water was carefully eliminated by paper tissue , and all samples were analyzed within 14 days of storage at room temperature (0, 7, and 14 days) (28).

Microbial tests. According to the procedures described by Erturk and Picha (29), enumeration of microorganisms and preparation of media and diluents were conducted. In this regard, aseptically cut potatoes, amounting to 50 grams, were randomly selected and homogenized with 450 mL of 1% peptone water using a mixer. The target microorganisms were counted immediately after the CAP pretreatment, on 7 and 14 days of storage. It's worth mentioning that the control samples were not subjected to CAP pretreatment. Nevertheless, it was kept in the same conditions as the non-control samples.

In this regard, Logarithmic dilutions were prepared and spread onto acidified PDA plates. Three plates per dilution were then incubated at 22 ± 1°C for 5 days to encourage yeast and mold growth. Afterward, appropriate logarithmic dilutions were applied onto PCA plates. Similarly, three plates were designated for each dilution. These plates were subjected to incubation under two specific temperature conditions: 32 ± 1°C for 48 h to quantify mesophilic microorganisms, and 7 ± 1°C for duration of 10 days to evaluate psychrotrophic microorganisms. Following the incubation period, colonies were visually counted using a magnifying glass. The results were presented as colony-forming units (CFU) per gram of sweet potato tissue.

Statistical analysis. Statistical analysis employed a fully randomized factorial design. Results were expressed as mean ± SD. One-way analysis of variance (ANOVA) determined significant differences, while Duncan's test assessed mean differences. SPSS (version 32) conducted statistical assessments, with graphical visualizations generated using Excel 2013.

RESULTS

Fig. 1 illustrates the influence of CAP on mesophilic and psychrotrophic bacteria populations during storage for four different groups: the control sample and the three samples pre-treated with cold plasma. As can be seen, bacterial population in all pre-treated samples was significantly lower than the control (P<0.05). In terms of fungal growth, Fig. 2 presents data regarding

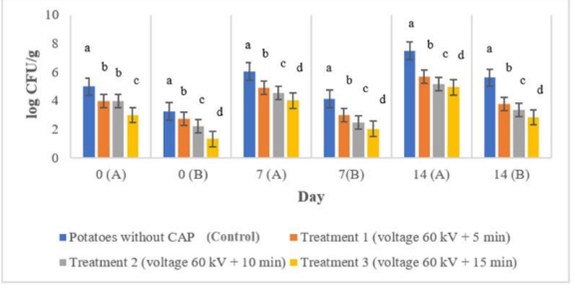

Fig. 1. Comparison of the logarithm count of mesophilic bacteria (log CFU/g) (A); and psychrophilic bacteria (log CFU/g) (B) in raw potato slices pretreated with CAP and the control during 14 days of storage. Different lowercase letters in in each day indicate a significant difference (P<0.05). Data are means ± SD.

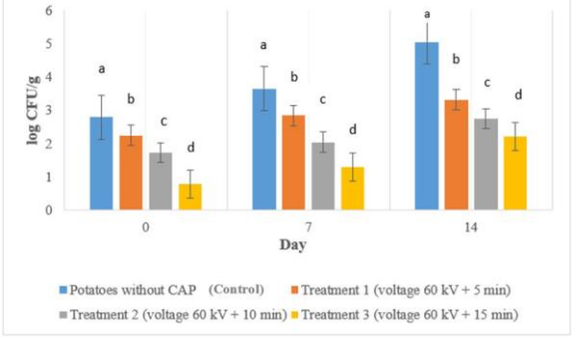

Fig. 2. Comparison of the logarithm count of mold and yeasts (log CFU/g) in raw potato slices pretreated with CAP and the control during 14 days of storage. Different lowercase letters in each day indicate a significant difference (P<0.05). Data are means ± SD.

the logarithm population of molds and yeasts within the tested sample. Initially, the microbial populations of mesophilic and psychrotrophic bacteria in the control samples were approximately 5 ± 0.07 CFU/g and 3.26 ± 0.02 CFU/g, respectively. However, by the 14th day of storage, these counts reached to 7.5 ± 0.12 CFU/g and 5.62 ± 0.17 CFU/g, respectively. With respect to the pretreated samples, such values for mesophilic and psychrotrophic bacteria in 5, 10 and 15 min pretreated samples reached to 5.7 ± 0.05 CFU/g, 5.16 ± 0.09 CFU/g, 4.95 ± 0.08 CFU/g and 3.78 ± 0.09, 3.37 ± 0.13 and 2.85 ± 0.09 CFU/g respectively, throughout the storage period.

DISCUSSION

In this study, the effect of CAP pre-treatment (precisely 60 kV voltage, exposure time 5, 10 and 15 min) was evaluated on microbial load of raw potato slices. Regarding bacterial growth, specifically mesophilic and psychrophilic types, the study revealed fluctuations during a 14-day storage period. It is important to highlight that the populations of both mesophilic and psychrotrophic bacteria in the pretreated samples were 1 to 3 times lower than those in the control ($P<0.05$). The statistical analysis demonstrated significant effects of the interaction between treatment and time (time × treatment) on the bacterial population ($P<0.05$). Notably, the selected voltage (60 kV) and the extended duration of cold plasma exposure (15 min) resulted in a more pronounced impact on the bacterial populations. The decline in the bacterial populations within the CAP-pretreated sample can be attributed to the generation of reactive oxygen and nitrogen species during plasma exposure (20, 30-32). These species are capable of interacting with bacterial lipopolysaccharides and peptidoglycans, resulting in molecular structure damage through the disruption of CON, COO, and COC bonds. This ultimately leads to the destruction of the bacterial cells. In this regard, several research findings suggest that prolonging the duration of plasma application results in an elevation of reactive species levels. The extended treatment time enhances the contact between microorganisms and these reactive species, ultimately resulting in microbial inactivation. In terms of fungal growth, as can be seen, there was a significant difference ($p<0.05$) in the logarithm counts of mold and yeast among pretreated samples and the control. Even though the logarithmic counts of mold and yeast demonstrated an increase over time, the prolonged exposure to cold plasma led to a significant reduction in the population of microorganisms ($p<0.05$). Consequently, the logarithmic counts of mold and yeast for the control (without CAP pretreatment) and treatment 3 (voltage 60 kV + 15 min) on day 14 were approximately 5.05 ± 0.27 CFU/g and 2.22 ± 0.07 CFU/g, respectively. In this regard, Pankaj et al. (33) demonstrated that exposing mold and yeast to cold plasma led to a reduction in their populations. The researchers attributed this reduction to the destruction of the DNA within the mold and yeast cells due to the effects of plasma reactive species (33). These reactive species can cause damage to the genetic material of microorganisms, ultimately leading to their inactivation. Additionally, the study observed that as the treatment time with cold plasma increased, the effectiveness of mold and yeast population inactivation also increased. This suggests that longer exposure to cold plasma resulted in more significant reduction in the populations of these microorganisms, which is in good agreement with our finding. Similarly, Herceg et al. (34) reported comparable results regarding the effect of cold plasma treatment time on bacterial and mold population. However, the intensity of inactivation and reduction in the population of aerobic and psychrophilic bacteria is much higher than in the population of mold and yeast. This difference can be linked to the thick structure of the cell wall of fungi compared to the peptidoglycan membrane of bacteria. Amini et al. (35) explained that fungi have a structure comprising elements like chitin, cellulose fibrils, and a polysaccharide matrix. These components enhance the cell wall toughness, leading to reduced DNA harm in fungi. Similar findings were noted by Nishime et al. (36) , who reported a more noticeable drop in the population of Gram-negative bacteria compared to *Candida albicans* mold when exposed to cold plasma treatment. They indicated that the thickness and hardness of fungi cell walls play a significant role in their resistance to plasma irradiation.

CONCLUSION

In the current study, the impact of cold plasma pretreatment on microbial populations of raw potato slices was investigated. The results clearly showed

that cold plasma is highly effective in reducing microbial growth over a 14-day storage period, especially when compared to the untreated samples. Additionally, a noticeable decrease in the populations of mesophilic and psychrotrophic bacteria, as well as mold and yeast, was observed with longer cold plasma exposure time (from 5 to 15 min) and this reduction was statistically significant compared to the control ($p<0.05$). Indeed, the most substantial reduction in bacterial and fungal counts achieved through cold atmospheric plasma treatment at 60 kV for 15 min. These findings underline the potential of cold plasma as a robust method for reducing microbial contamination in raw potato slices. The study not only highlights the promising role of cold plasma in enhancing food safety but also emphasizes the importance of adjusting treatment parameters to achieve the best results in microbial load reduction. However, further research is recommended, involving different voltage levels, varying time durations, and the inclusion of other types of vegetables.

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