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Investigation of microbial load of industrial dried vegetables based on cold plasma

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Abstract

Nowadays, food industry experts consider the use of cold plasma method as a non-thermal method for reducing microbial load and cold sterilization. In this study, the antimicrobial effect of cold plasma was considered in order to provide microbial safety. The aim of this study was to investigate the effect of cold plasma on the work cycle (8, 12 and 16) percent and different times (2.5, 5 and 10 minutes) to evaluate the microbial properties of cold plasma treatments. The results of this study showed that with increasing the duration of effectiveness with cold plasma, the growth rate of bacteria such as total microorganisms, mold and yeast, Escherichia coli, and Enterobacteriaceae decreased significantly, and was negative in Staphylococcus aureus ($P < 0.05$). It should be noted that the microbial quality of cold plasma-treated treatments was much lower than the control treatment. In general, the results of this study showed that the use of non-thermal cold plasma in dried vegetables maintains and improves the nutritional value without adversely affecting the treatments, and is also a good alternative to thermal depollutionizing methods.

Keywords: Cold sterilization, Cold plasma, Non-thermal, Antimicrobial

Introduction

Since vegetables are rich in vitamins, minerals and antioxidant compounds and have high concentrations of moisture and low fat, they reduce the risk of cardiovascular disease and cancer. For this reason, ensuring the health of this valuable food is very important in order to maintain and ensure the health of people. Drying vegetables as one of the oldest methods in order to preserve the food by removing its moisture while maintaining its nutritional value is very important; however, the deformation and volume reduction in most cases is a negative feature. Glass, paper, aluminum, tin-free steel, and plastics (polyester, polystyrene, polyethylene, and polyamide) are mainly used in food packaging. Polyethylene has a high market share due to its low weight, high impermeability, high strength, hydrophobic nature and low surface energy [Alexander and Smaje, 2008]. In order to disinfect foods, in various types of food packaging, methods such as oven, autoclave, dry heat, ethylene oxide and gamma radiation, etc. can be used [Dave and Ghaly, 2011]. In recent years, plasma has been used to kill bacteria and microorganisms [Roth et al, 2000]. Today, cold plasma or non-thermal plasma is used for disinfection [Napartovich, 2001]. Generally, non-thermal plasma is produced for disinfection at low pressure and atmospheric pressure, but due to the large volume of plasma at low pressure, it is expensive, therefore, more attention was paid to the production of plasmas at atmospheric pressure [Laroussi et al, 2003]. The plasma phenomenon causes the formation of an adiabatic system 11 with a high content of kinetic energy at temperatures below 70 ° C [Schluter et al, 2013]. Cold plasma plays an effective role as an antimicrobial technology developed to sterilize and inactivate microorganisms. This technology has been the focus of the food industry since 1990 and is used as a method of using ionized gases produced at room temperature and atmospheric pressure [Noriega et al, 2011; Fernandez et al, 2012]. Cold plasma also emits charged and energetic particles, radicals and photons, and by breaking covalent bonds, it causes numerous chemical reactions that are of great importance in various applications of technology [Arjunan et al, 2015].

Materials

This research was conducted in one of the companies in the Golestan province, Iran. To prepare the raw materials of dried vegetables from Golestan market and chemicals of potassium chloride and sodium acetate buffer and microbial culture medium (Slentzobarleti, streamide, sulfhydryl agar, Y.G.C and brilliant green broth) were purchased from Merck Company in Germany. Atmospheric cold plasma containing argon gas (99.997% purity) was examined during 4, 6, and 10 min flow times. Cold plasma electrode with a maximum voltage of 1 kV connected to the power supply at an approximate frequency of 6 kHz was examined. The quartz chamber was considered as a dielectric barrier and the human diameter containing the sample and the diameter of the end of the high voltage probe were considered to be about 4.5 and 0.6 cm, respectively. They were treated with cold plasma for 2.5, 5 and 10 minutes.

Method

First, some dried vegetables taken from the market, approved by the national standard of Iran, were examined based on three treatments (packaging of dried vegetables under cold plasma process) in three repetitions in a specified time period, each treatment with five samples, were examined compared with fifteen packageing of dried vegetables without cold plasma as the control group, and finally, the microbial tests, such as enterococci (Slentzobartli culture medium) according to Iranian National Standard No. 2198; Staphylococcus aureus according to Iranian National Standard No. 03-6806; Escherichia coli in accordance with the national standard of Iran No. 11166; Mold and yeast (culture medium) Y.G.C in accordance with the Iranian National Standard No. 2-10899 and the total count of microorganisms with the Iranian National Standard No. 4207, were applied.

Counting mold and yeast

In accordance with Iranian National Standard No. 2-10899, Y.G.C culture medium was used to isolate and count mold and yeast extract of glucose agar to separate yeasts. Several samples were prepared in 1-10 and 2-10 dilutions and about 10 grams of the sample was ground under sterile conditions mixed with 9 milliliters of physiological serum. To prepare 2-10 dilutions with pipette, 1 milliliter of 1-10 dilution was taken. Under sterile conditions, 9 milliliter of physiological serum was poured into a test tube and the resulting mixture was placed on a shaker for 15 seconds in order to uniform the samples. Then, 1 ml of the sample was poured into a plate and the culture medium was chloramphenicol dextrose. We added it and moved it in an 8-shaped manner , which, after solidification of the culture medium for 72 hours, was subjected to the incubation process at 25 ° C, and finally, the number of colonies per milliliter of the sample was counted.

Escherichia coli count

11 grams of green broth brilliant medium were dissolved in 250 milliliters of distilled water; The Erlenmeyer lid was sealed with foil and cotton and placed on the flame. After boiling, four sterile test tubes were selected and the Durham tube was placed upside down inside them. Then, 11 milliliters of Green Broth Brilliant culture medium was poured into the tubes and the lids of the tubes were closed and placed in an autoclave with physiological serum. The Erlenmeyer door was sealed with cotton and foil and placed on the flame. In the third stage, 5 grams of the sample was poured into a porcelain mortar and gently shaken. During the addition of the sample, 45 milliliters of physiological serum was added to the treatment, and after preparing the desired suspension, three test tubes were selected, in each of which, 9 milliliters of serum was added. Then 1 milliliter of the sample was taken from inside the mortar and poured into tube number one and gently shaken, and about 1 milliliter was taken from the first sample and added inside the second tube and gently shaken. From the second tube, 1 milliliter of the sample was taken by a pipette, and added to the third tube and shaken gently. In the fourth step, after preparing the dilutions and numbering, 1 milliliter of each dilution was added into the tubes containing BGB culture medium. The tubes were numbered, and at the end, 1 mililitter of the sample was poured into the plate and the bottom of the plate was filled with violet red Bayer agar culture medium. The watch was incubated at 31°C, and in case there was gas inside the Durham tube and a colony in the plate, the presence of coliform was confirmed.

Staphylococcus aureus count

In this study, about 9 cc of the test sample was incubated with a dilution of 1-10 to 9 cc of Giulietti Canton broth
culture medium at 37 ° C for 48 hours. Based on different species of Staph, the existing tellurite was reduced in the environment and turned into tellurium, causing the environment to turn black. The broth was then cultured on Parker board from the Giulietti canton medium and incubated for 37 hours at 37°C. In the presence of Staphylococcus aureus, black colonies were observed on this medium.

Enterobacteriaceae count

About 1 gram of the sample was transferred to two plates in liquid glucose culture medium and after inoculating the plates and adding 15-20 milliliters of Slantz and Bartley culture medium containing 1% TTC at 45 ° C, it was cultured as a plate (temperature 37- 35 ° C for 24-48 hours) and incubated. Pink, purple to red colonies were counted and a certain number of colonies were selected for confirmatory scoliosis hydrolysis tests for the absence of enterococci and were measured on a unit basis based on CFU per gram or ml of the sample.

General enumeration of microorganisms

In this assay, purplate culture method was prepared with a dilution of 1-10 and was cultured with physiological serum in plate count agar medium and then incubated at 30 ° C for 48-72 hours and the total number of microorganisms was counted.

Statistical analysis

In order to investigate the effect of cold plasma treatment, in three time intervals (2.5, 5, 10) and work cycle (8, 12 and 16%), on reducing microbial load, shelf life and nutritional value of samples using a complete design Duncan's multiple range test was used at 5% probability level and analysis of variance, then the results were plotted using Excel 2016 software.

Results Discussion

Antimicrobial effect of cold plasma on mold and yeast

Findings from the study of mold and yeast count in dried vegetable treatments (marjoram, mint, lemongrass, lemon and

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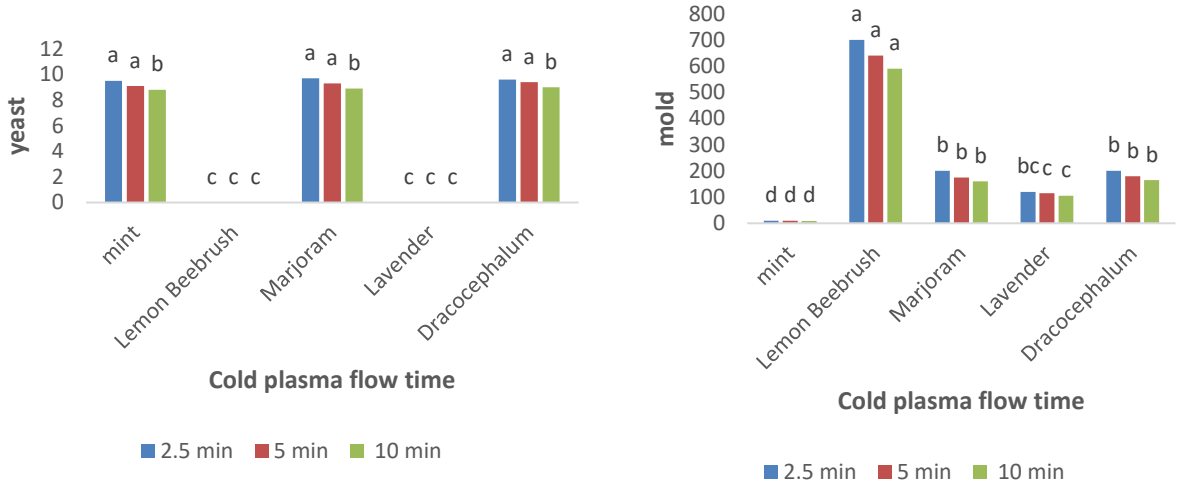
lavender) treated with cold plasma compared to the control treatment showed in Figures 1 that statistically, The results show that with increasing storage time (working cycle), the amount of mold and yeast, the was significantly decreased (P≥0.05). Some researchers stated that cold plasma treatment is effective in inhibiting Aspergillus flavus mold [Suhem et al, 2013]. Ohkawa et al. also reported that the cold plasma process has a detrimental effect on the cellular structure of Candida albicans [Ohkawa et al, 2006]. Other researchers also found that cold plasma treatment over time cycles (8, 12 and 16%) and time (2.5, 5 and 10 minutes) reduced the amount of inoculated yeasts such as Candida albicans, Crocus and Cryptococcus neoformans and Aspergillus Tereus mold [Venezia et al, 2008]. thus, based on the results, it can be concluded that plasma species interact with food and inactivate or kill microbial cells.The interaction between plasma and microbial cells types is a key factor in the effect of cold plasma. The oxidative effect of plasma types leads to the destruction of the microbial cell wall. Moisture quantity plays an important role in microbial demotion. Increased moisture increment the activity of plasma species on microbial cells. ergo, humid microorganisms get destroyed more quickly than drier microorganisms [Dobrynin et al, 2009].



Figures 1. Comparison of the effect of cold plasma treatment time on microbial reduction of mold and yeast.

Antimicrobial effect of cold plasma on Escherichia coli

Different studies have shown that the use of high voltage electrical discharge plasma in dried vegetables caused a change in Escherichia coli morphology through damage to their cell wall (Figures 2) [Yannam et al, 2018]. The results showed that the percentage of Escherichia coli before and after cold plasma treatment increased the logarithmic cycle by increasing the time of 10 minutes in the work cycle (8, 12 and 16%). In a study, researchers reported that the effect of cold plasma on Escherichia coli and Staphylococcus aureus increased the number of Escherichia coli colonies less than that of Staphylococcus aureus at 30, 60, 90, and 120 minutes [Xingmin et al, 2006]. The rupturing and in result of inactivation of E. coli in reaction to plasma treatment might be due to generated reactive oxygen and nitrogen species. E. coli is a gram negative bacteria covered by a thin layer of peptidoglycan and an outer membrane of lipopolysaccharide. The reactive oxygen (ROS) and nitrogen species (RNS) generated due to plasma treatment that can respond with both lipopolysaccharide and peptidoglycan, according as a result, harmful the molecular structure by breakdown COO, CON, and COC bonds that resulted in leakage of cellular debris of E. coli [Chung et al, 2013; Han et al, 2016; Yusupov et al, 2013]. beside, these gas species can also reason lipid peroxidation of lipopolysaccharide membrane resulting in dissociation of cell cover [Joshi et al, 2011].

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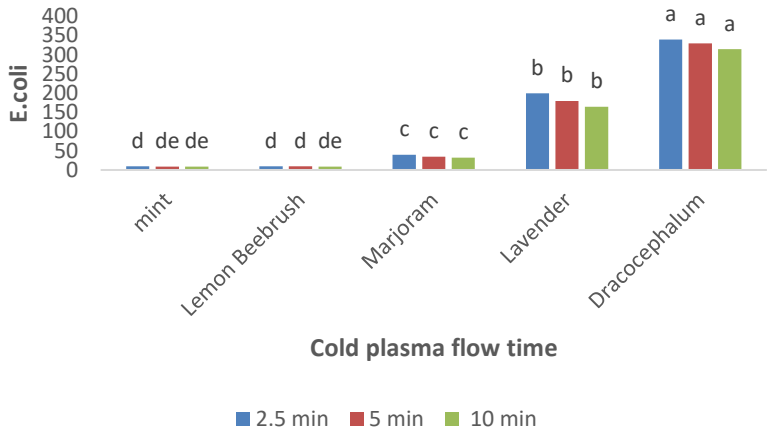

Figures 2. Comparison of the effect of cold plasma treatment time on microbial reduction of Escherichia coli.

Antimicrobial effect of cold plasma on Staphylococcus aureus

The results of this study showed that the amount of Staphylococcus aureus bacteria was negative by increasing 10 minutes in the work cycle (8, 12 and 16%) during the storage period of Staphylococcus aureus bacteria. The researchers found that during 25 minutes of cold plasma at room temperature, the amount of Staphylococcus aureus and Escherichia coli was reduced over the work cycle [Roth et al, 2000]. Zuizina and other reaserchers understand that application of cold plasma for Gram-positive bacteria which have the thicker cell wall than Gram-negative bacteria, is differnt and the thicker cell wall of Gram-positive bacteria proves to be a barrier to the application of cold plasma [Ziuzinaet al, 2014]. the effectiveness of cold plasma in inactivating microbial load on fresh produce is subject to type of microbial contamination. In addition, the efficacy of microbial inactivation is as well as indirectly proportional to the surface of roughness, initial microbial load and the maturity or growth stage of the contaminating microorganisms [Bhide et al, 2017].

Antimicrobial effect of cold plasma on Enterobacteriaceae

The results of Enterobacteriaceae count in dried vegetable treatments (mint, marjoram, lemongrass, lemon and lavender) treated with cold plasma in comparison with the control treatment showed that, with increasing time and work cycle (8, 12 and 16%), the amount of bacteria Enterobacteriaceae was significantly reduced ($P \geq 0.05$). (Figures 3). Tappi and other researchers report that On fresh cut melons treated with plasma generated using atmospheric air, mesophilic and lactic acid bacteria showed highest reduction (3.4 and 2 log10, respectively). also relate that During storage, pieces of melons treated for 15 min on each side show the greatest increase in shelf life [Tappi et al, 2016]. Bhide et al, in their research found that the efficacy of atmospheric air begatten plasma in inactivating Enterobacter spp on cantaloupe peel decreased as the quality of the peel increased [Bhide et al, 2017].

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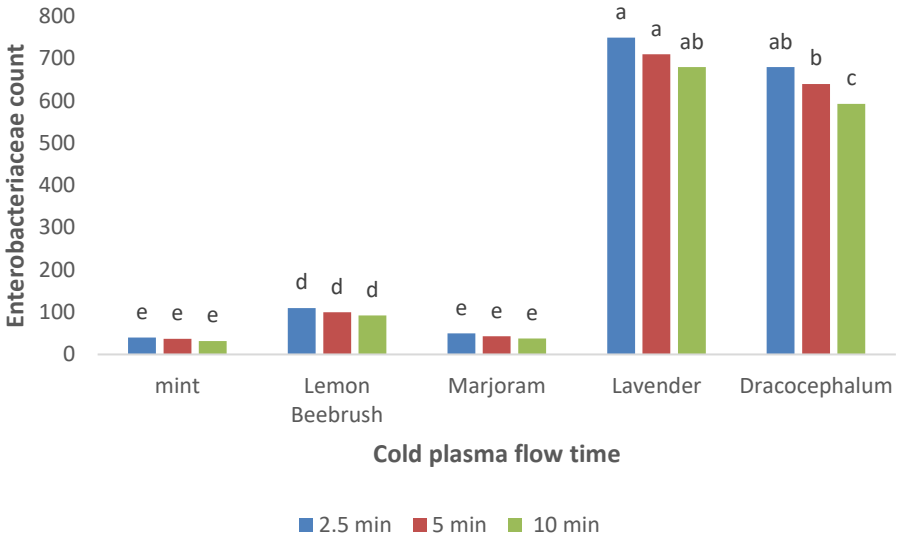
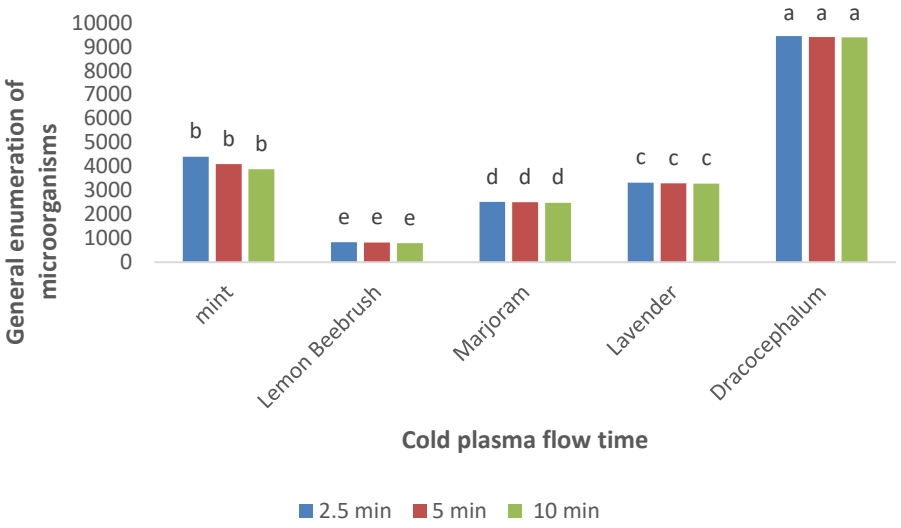


Figures 3. Comparison of the effect of cold plasma treatment time on microbial reduction of Enterobacteriaceae

Antimicrobial effect of cold plasma on the total count of microorganisms

Non-thermal methods such as cold plasma, are able to reduce the microbial load of the product and provide health and safety for people. The statistical results of comparing the average total count of microorganisms treated with plasma in comparison with the control treatment showed that, with increasing the applied time, the total count of microorganisms decreases significantly (Figures 4). Therefore, with increasing plasma treatment time (2.5 to 10 minutes) in the work cycle of 8 to 16%, a significant decrease is caused ($P \geq 0.05$). Moisan et al. pointed out the importance of free radicals and the effect of ultraviolet rays and charged particles such as ions and electrons on plasma and their antimicrobial effects [Moisan et al, 2001]. Hydroxyl radicals are also able to break down the oxidation of unsaturated fatty acids in the cell wall and interfere with the oxidation of proteins and act as an active antimicrobial compound [Surowsky et al, 2014].



Figures 4. Comparison of the effect of cold plasma treatment time on microbial reduction of the total enumeration of microorganisms.

Conclusions

The use of cold plasma, due to less environmental pollution in killing germs and sterilizing living tissues, has been highly welcomed as a method in the last decade in the world with the aim of removing pollution [32]. In this study, the effect of head plasma on the

microbial quality of dried vegetables (mint, marjoram, lemongrass, lemon and lavender) was investigated over and different time and work cycles. The results showed that by increasing the time and work cycles of cold plasma treatment, the total microbial load of mold, mold and yeast, Escherichia coli, Staurus and Enterobacteriaceae was reduced, which was more effective at longer times of the lower work cycle. Therefore, it can be said that the use of plasma treatment in dried vegetables increases its nutritional value without having an adverse effect on the quality characteristics of the product. Furthermore, a significant effect in reducing the pollution of cold plasma treatment was able to reduce the microbial load of the product under the approved national standard of Iran.

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