Research Journal of Microbiology

ISSN 1816-4935

Academic Journals Inc.

www.academicjournals.com

OPEN ACCESS Research Journal of Microbiology

ISSN 1816-4935
DOI: 10.3923/jm.2016.42.46

Research Article
Black and Green Tea Decontamination by Cold Plasma

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Abstract

This experimental study is the first study conducted to black tea and green tea decontamination by cold atmospheric pressure plasma jet. This study shows that the plasma jet system is effective in inactivation of the pathogenic microorganisms of black and green tea and the inactivation of the considered strains were greatly affected by treatment time, initial concentration and kind of microorganisms. Treatment of 3 and 5 min led to complete inactivation of *E. coli* and coliform cells on black tea and complete decontamination of mold and yeast was achieved after 7 min plasma jet treatment time while complete reduction of *E. coli* and coliform cells of green tea were achieved after 4 and 5 min cold plasma treatment, respectively. Yeast and mold were completely inactivated after 7 min cold plasma treatment. In order to determine quality changes of tea samples during plasma treatment, the effect of plasma jet on caffeine, color and total phenolic content of black and green tea were evaluated. There were no significant changes in caffeine of both tea samples after 7 min plasma jet treatment. The initial total phenolic content of black tea and green tea was 10.84 and 14.94 g GAE/100 g, respectively. In both tea samples, total phenolic content was slightly increased after 5 min plasma treatment. In both tea samples 7 min plasma treatment had no significant effect in the L* (darkness to lightness), a* (green to red) and b* (yellow to blue) value of tea samples.

Key words: Cold plasma, black and green tea, decontamination, physico-chemical parameters of tea, *E. coli*

Received: September 07, 2015 **Accepted:** October 22, 2015 **Published:** December 15, 2015

Citation: Maryam Amini and Mahmood Ghoranneviss, 2016. Black and Green Tea Decontamination by Cold Plasma. Res. J. Microbiol., 11: 42-46.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The tea plant is cultured all around. The quality of tea is different in countries and it depends on climate, farming techniques and industrialization (Saito *et al*., 2007). Teas is divided into three categories based on the tea manufacturing (fermentation) process: Green tea (unfermented), oolong tea (partially fermented) and black tea (fully fermented) (Kim *et al*., 2011). Black tea is one of the most popular beverages due to its refreshing effect and unique aroma. The black tea aroma consists of aldehydes, alcohols, terpenes, nitro compounds, hydrocarbons and linalool. Green teas are preferred by the drinkers due to the promising health benefits for example: insulin-enhancing activity (Anderson and Polansky, 2002), anti-inflammatory capacities (Saito *et al*., 2006), protection against cardiovascular (Sano *et al*., 2004) and brain (Suzuki *et al*., 2004) diseases and antimicrobial. Contamination of tea with pathogenic microorganisms is a potential risk for health. Cold plasmas have recently received considerable interest for their ability to invitation of microorganisms. Cold plasmas are environment friendly and are more effective than conventional oxidants and disinfectants. Cold plasmas are an efficient source of electrons, ions, UV light, electric field and free radicals, depending on the discharge conditions. Depending on the type of set up and input gas and power, various types of chemical reactions can be initiated and several primary and secondary species can be formed by plasmas. Cold plasma has been studied for variety of food products (Kim *et al*., 2014; Ziuzina *et al*., 2014; Bermudez-Aguirre *et al*., 2013). But decontamination of herbs and powder by cold plasma is rare. Kim *et al*. (2014) applied cold plasma on red pepper. They reported that 2.5-0.3 log spores/g reduction of A. flavus was achieved after 20 min cold plasma treatment with nitrogen at 900 W.

Although, cold plasma is considered to generate pure surface effects, the effect of plasma quality and safety of object is important to validate cold plasma as a new preservation method.

Total polyphenol content is directly correlated to the quality of the product (Lin *et al*., 1998). Caffeine is an important quality parameter of tea and it is contributed to tea flavor. Color parameter analysis is useful to characterize the quality of products. The aims of this study are to evaluate the effect of cold plasma on (1) Decontamination of black tea and green tea, (2) Caffeine, total phenolic content and color of black tea and green tea. This is the first study that conducted to cold plasma decontamination of tea.

MATERIALS AND METHODS

Sample preparation: Two hundred grams of newly dried of black and green tea were purchased separately from a production centers located in Lahijan, Gilan, Iran.

Microbial analysis: At first 20 g of the tea samples were analyzed using standard procedures in order to determine bacterial and fungal species. All bacterial and fungal isolation were according to the recommendations prepared by the Institute of Standards and Industrial Research of Iran (ISIRI) as follow: ISIRI: 2946 for *Escherichia coli*. ISIRI: 1116 and 9236 for coliform species identification. ISIRI: 997 for mold and yeast species identification.

Microbial enumerating: Microbial analysis of coliform, *E. coli*, mold and yeast was carried out for evaluating the effect of plasma jet on decontamination of black and green tea. About 120 g of each tea samples was treated by the plasma jet at different treatment time and 20 g of each tea sample was kept untreated as a control. Treated and untreated tea sample was diluted with 90 and 40 mL sterile peptone water. Total coliforms and *Escherishia coli* were determined by adding 1 mL of each suspension to MacConkey Broth (Oxid Comp., Basingstoke, Hant, UK). Total mold and yeast counts were determined by adding 1 mL of each suspension to Malt Extract Agar (MEA) medium (Difco-Labs, Detroit, Michigan, USA) after incubation at 25°C for 72 h.

Experimental set up: The plasma jet consisted of a pyrex tube and power electrode (copper wire) which was wrapped around the glass tube as nozzle. The power of electrode was driven by a 10 kHz pulsed DC 10 kV high voltage power supply. For evaluating the effect of the plasma jet on food, the feeding gases were 99.999% pure argon (Ar) with 1 L min^{-1} gas flow rate and distance between the samples and the nozzle tip was 1.5 cm that was kept unchanged. The 5 g of sample was put on petri dish and treated by cold plasma.

Determination of caffeine levels: Caffeine was determined by HPLC using the technique reported in ISO (2002).

Determination of total phenol levels: The total phenolic compounds were estimated by the Folin-Ciocalteu reagent. About 50 µL of extracts, 10 mL of distilled water and 1 mL of Folin-Ciocalteu reagent (Sigma-Aldrich, St. Louis, EUA) were mixed in a 20 mL volumetric flask. The mixture were placed at room temperature for 3 h. After

that 8 mL of sodium carbonate (75 g L^{-1}) was added. The final solution was adjusted to 20 mL by adding distillated water and the absorbance at 765 nm (Singleton and Rossi Jr., 1965) was measured. Gallic acid was used as standard and the total phenolics content was expressed as one gram of Gallic Acid Equivalent (GAE) per gram of sample.

Determination of color of tea: The color values the L* (darkness to lightness), a* (green to red) and b* (yellow to blue) of tea samples were measured with Hunter Lab spectrophotometric colorimeter (D25 DP9000, Hunter Associates Laboratory, Inc., Reston, USA) calibrated with black and white reference standard.

Statistical analysis: SPSS software version 22.0 (SPSS Inc., Chicago, IL, USA) was used to perform statistical analysis. Average values from triplicate experiments were obtained and one-way analysis of variance (ANOVA) followed by Duncan's test was performed to determine the differences in mean values of achieved data from different treatment times. Statistical differences were considered as significant at $p<0.05$.

RESULTS AND DISCUSSION

Table 1 shows the reduction of microorganisms after the plasma jet treatment as a function of treatment time. The initial concentration of *E. coli,* coliform, mold and yeast of black tea sample were 3.2, 4.5, 3.00 and 3.1 log/g, respectively. The results show that 1 min argon plasma jet treatment led to *E. coli* and coliforms cell reduction by 1.2 log. Increasing treatment time led to increase inactivation rate and 3 and 5 min treatment time led to complete inactivation of *E. coli* and coliform on black tea, respectively. The reduction of mold and yeast of black tea is shown in Table 1. As can be seen from the results, less reduction of mold and yeast was achieved after plasma treatment and 4 min plasma jet treatment led to 2 and 2.1 log reduction of mold and yeast on black tea, respectively. The complete decontamination of mold and yeast was achieved after 7 min plasma jet treatment time. Table 1 shows the reduction of microorganisms after the plasma jet treatment as a function of treatment time. The initial concentration of *E. coli,* coliform, mold and yeast of green tea sample were 4.00, 4.50, 3.10 and 3.30 log/g, respectively. The results show that the total inactivation of *E. coli* cells of green tea was achieved after 5 min cold plasma treatment while 4 min cold plasma treatment cause complete inactivation of *E. coli* cell of black tea. Fernandez *et al.* (2012) reported that the initial concentration of microorganisms on foods plays an important role in the cold plasma inactivation rate. Cold plasma generates electron discharge without temperature increase which consists of highly energetic species that break covalent bonds and initiate many chemical reactions. In other hand UV photons, electrons, ions, free radicals are generated in plasma inhibit microorganisms effectively (Fernandez *et al.*, 2012). Penetrating reactive species through cell wall or membrane cause damage by reacting with some components such as membrane lipids, proteins and nucleic acids and cause surface erosion in the cell membrane. The formation of surface erosion is because of entering of the reactive species into cells, which can enhance microbial inactivation (Gallagher *et al.*, 2007). Reactive species such as Reactive Nitrogen Species (RNS) and reactive oxygen spices, UV photons, electrons, free radicals, cause inactivation of bacteria inoculated on tea. For example: Bombardment of the cell wall cause disrupts bacteria cell membranes, denature proteins and damage bacterial DNA. Mendis *et al.* (2000)

Table 1: Reduction of *E. coli*, coliform, mold and yeast after the cold plasma treatment

	Number of survivors (CFU)							
	Treatment time (min)							
	0	1	2	3	4	5	6	7
Black tea								
E. coli	3.20±0.26^{a}	2.00±0.35^{b}	0.80±0.54^{c}	0.00	0.00	0.00	0.00	0
Coliform	4.50±0.68^{a}	3.33±0.60^{b}	2.40±0.42^{c}	1.85±0.85^{d}	0.60±0.23^{e}	0.00	0.00	0
Mold	3.00±0.23^{a}	2.40±0.43^{b}	1.80±0.23^{c}	1.60±0.35^{d}	1.00±0.25^{e}	0.80±0.20^{f}	0.50±0.10^{g}	0
Yeast	3.10±0.23^{a}	2.70±0.33^{b}	2.00±0.3^{c}	1.60±0.1^{d}	1.00±0.5^{e}	0.70±0.23^{f}	0.30±0.15^{g}	0
Green tea								
E. coli	4.05±0.29^{a}	3.20±0.45^{b}	2.00±0.42^{c}	0.80±0.52^{d}	0.00	0.00	0.00	0
Coliform	4.50±0.50^{a}	3.60±0.40^{b}	2.90±0.40^{c}	2.10±0.33^{d}	1.10±0.54^{e}	0.00	0.00	0
Mold	3.00±0.45^{a}	2.73±0.52^{b}	2.10±0.37^{c}	1.77±0.50^{d}	1.32±0.41^{e}	0.90±0.53^{f}	0.50±0.50^{g}	0
Yeast	3.30±0.31^{a}	2.81±0.32^{b}	2.45±0.10^{c}	1.80±0.50^{d}	1.30±0.33^{e}	1.00±0.35^{f}	0.65±0.40^{g}	0

Mean values followed by different superscript letters differ significantly at $p<0.005$, CFU: Colony forming unit

Table 2: Effect of cold plasma on tea components

		Treatment time (min)							
Tea contents	Tea samples	0	1	2	3	4	5	6	7
Caffeine of tea	Black tea	2.40±4.05[a]	2.40±3.3[a]	2.48±5.26[a]	2.43±2.8[a]	2.40±6.03[a]	2.46±5.22[a]	2.41±9.13[a]	2.44±5.76[a]
(g/100 g dm)	Green tea	1.90±3.07[a]	1.90±3.14[a]	1.80±5.47[a]	1.83±2.90[a]	1.87±4.43[a]	1.91±0.05[a]	1.90±0.19[a]	1.91±7.10[a]
Total phenol	Black tea	10.77±0.16[a]	10.80±3.37[a]	10.80±1.14[a]	10.79±9.23[a]	10.98±0.04[b]	11.21±7.03[b]	11.38±2.02[c]	10.84±4.05[a]
(g GAE/100 g)	Green tea	14.94±7.10[a]	14.94±0.46[a]	14.92±5.00[a]	14.94±1.12[a]	14.93±3.01[a]	15.00±0.03[b]	15.91±1.30[c]	16.02±4.14[c]
Color									
a*	Black tea	2.10±1.65[a]	2.10±5.35[a]	2.00±7.44[a]	2.09±3.26[a]	2.00±7.44[a]	2.08±5.12[a]	2.00±0.02[a]	2.10±0.5[a]
b*	Black tea	8.20±0.71[a]	8.30 ±0.02[a]	8.20±0.60[a]	8.22±8.14[a]	8.25±1.90[a]	8.32±0.17[a]	8.25 ±0.71[a]	8.30±4.12[a]
L*	Black tea	22.90±0.54[a]	22.10±23.0[a]	22.40±1.1[a]	22.70±4.2[a]	22.50±11.8[a]	22.70±0.1[a]	22.50±66.0[a]	22.60±3.4[a]
a*	Green tea	-1.10±2.10[a]	-1.10±0.2[a]	-1.10±5.50[a]	-1.10±7.41[a]	-1.10±23.1[a]	-1.10±6.54[a]	-1.10±9.00[a]	-1.10±3.20[a]
b*	Green tea	2.52±5.50[a]	2.54±3.10[a]	2.51±7.56[a]	2.50±0.08[a]	2.50±3.06[a]	2.56±4.55[a]	2.57±1.11[a]	2.50±4.60[a]
L*	Green tea	37.10±4.8[a]	37.10±5.4[a]	37.00±0.9[a]	36.90±7.23[a]	37.00±3.5[a]	37.10±0.11[a]	37.20±0.16[a]	37.10±4.8[a]

Mean values followed by different superscript letters differ significantly at p<0.005

suggested that charged particles may play an important role in the rupture of outer cell membranes. Our results show that reduction of bacteria is more than fungal cell and it is because of thicker fungal cell wall structure. This cell wall is different from peptideglycan of bacterial membrane and consists of some compounds such as: chitin, cellulose fibrils and a polysaccharides matrix, which lead to more resistance of fungal cell to the stress. Despite bacteria fungal cells are eukaryotic cell that has a nucleus containing a diploid genome. These properties cause higher resistance to DNA damage. Although, several studies have demonstrated the microbial inhibition effects of cold plasma, to date there are no study conducted to decontamination of tea by cold atmospheric plasma jet for comparison. There are just few study conducted to effect of cold plasma on powder food or herb products. Kim *et al.* (2014) reported that 20 min cold plasma treatment at 900 W for 20 min cause inhibition of total aerobic bacteria in the red pepper powder by 1 log CFU/g.

Effect of cold plasma on caffeine content of black and green tea: The effects of the plasma jet on caffeine content of black and green tea, are shown in Table 2 ($p<0.05$). The initial concentration of caffeine content of black tea and green tea were 2.4±4.05, 1.9±3.07 g/100 g dm, respectively. No significant changes were observed in the caffeine content after 7 min plasma jet treatment with Ar gas. There is no study about effect of cold plasma on caffeine for comparison.

Effect of cold plasma on total phenolic content of black and green tea: The influence of different cold plasma treatment time on the total phenolic content of grean tea and black tea are shown in Table 2. Our results show that the initial total phenolic content of black tea and green tea were 10.84 and 14.94 g GAE/100 g, respectively. In both tea samples, total phenolic content was slightly increased after 5 min plasma treatment. Previous studies (Matan *et al.*, 2015; Kovacevic *et al.*, 2016) indicated that reported that cold plasma treatment led to increase in total phenolic content. Garofulic *et al.* (2015) reported that phenolic acid was increased by 15% after plasma treatment. They also reported that increase of total phenolic content depended on treatment time. Our results indicate that increasing treatment time led to increase in total phenolic content. Lee *et al.* (2009) found that the extent of total phenols was increased in gamma irradiated plants increase in total phenols may be because of larger phenolic compounds degradation into smaller compounds or release of phenolic compounds from glycosidic components (Harrison and Were, 2007). Radicals such as hydroxyl radicals and hydrogen atoms may break the glycosidic bonds (Fan and Mastovska, 2006). Our results show that plasma treatment led to negligible increase in total phenolic content of black and green tea. The free radicals generated in plasma may break the glycosidic bonds and produce monomers which increase the total polyphenolic content in plasma treated tea.

Effect of cold plasma on color of black and green tea: Effect of cold plasma treatment on color of black and green tea is shown in Table 2. The initial L*, a* and b* value of black tea was 22.6±3.4, 2.1±0.5, 8.3±4.12, respectively. The initial L*, a* and b* value of green tea were 37.1±4.8, -1.1±2.1, 2.5±4.6, respectively. The results show that in both tea samples 7 min plasma treatment had no significant effect in the L*, a* and b* value of tea samples. Gurol *et al.* (2012) reported that 9 min corona discharge treatment had no effect on color of milk. Kim *et al.* (2014) reported that cold plasma treatment had no effect on a*, b* and L* value of red pepper. They also reported that the temperature increase during cold plasma treatment is too small to affect the color of the powder.

CONCLUSION

In this study, plasma jet system was examined for its ability to decontaminate *E. coli*, coliform, mold and yeast on black tea and green tea. It was found that this plasma system significantly reduced the population of *E. coli,* coliform, mold and yeast in black and green tea. Moreover decontamination rate depend on treatment time and kind of microorganisms. Treatment of 3 and 5 min treatment time led to complete inactivation of *E. coli* and coliform cells on black tea and complete decontamination of mold and yeast was achieved after 7 min plasma jet treatment time while complete reduction of *E. coli* and coliform cells of green tea were achieved after 4 and 5 min cold plasma treatment, respectively. Yeast and mold were completely inactivated after 7 min cold plasma treatment. There were no significant changes in caffeine of both tea samples after 7 min plasma jet treatment. In both tea samples, total phenolic content was slightly increased after 5 min plasma treatment. Plasma treatment of 7 min had no significant effect in the L*, a* and b* values of black tea and green tea samples.

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