CHAPTER 4

Results and Discussion

4.1 Effect of NTP on degradation pesticide residues, physicochemical properties and sensory attributes of mango before and after plasma treatments

4.1.1 Chlorpyrifos

The effect of decontamination by NTP treatments with three different flow rates of Ar gas for 1, 5, 10 or 15 min on chlorpyrifos residues in mango surface are shown in Table 4.1. It can be seen that all the control and NTP conditions applied had an effect on reducing the pesticide. The initial chlorpyrifos concentration of sprayed mango was found to be 2.84 ± 0.2 ppm. The control sample which was the fruits covered with solution of pesticide (100 ppm) and soaked in normal distilled water for 15 min without any plasma treatment had 1.59 ± 0.37 ppm of pesticide concentration and decreased by 44.27 % from the original level. For NTP treated samples, the amount of chlorpyrifos after plasma treatments was ranged from 0.74 to 2.16 ppm with the reduction rate from 24.07 to 74.02 %. Among these set of NTP conditions, 5 L/min of Ar flux for 5 min gave the highest efficacy of removal chlorpyrifos. There was also high reduction rate of the pesticide more than 70 % observed at 5L_10 min and 8L_5min treatments. Except 2 L/min of Ar flow rate, the higher sanitizing capability was observed during the middle treatment period (5 or 10 min) rather than the early or the later one.

According to Codex Alimentarius Commission, FAO/WHO Food Standard Programme (2016), the Maximum Residue Limits (MRLs) of chlorpyrifos on spices, fruits and berries is 1 ppm. With the observed results, the quite safe levels of chlorpyrifos residues in mango which were 0.74, 0.85 and 0.78 ppm had been obtained after operating NTP treatments coded 5L_5min, 5L_10min and 8L_5min, respectively. Under selected NTP conditions, chlorpyrifos residues were significantly ($p \leq 0.05$)

decreased with the faster and more efficient rate than the control treatment using only water.

Table 4.1 Effect of NTP treatments on degradation of chlorpyrifos residues in mango

Flow rate of Ar gas (L/min)	NTP treatment time (min)	Test ID	Concentration (ppm)	Reduction rate (%)
Mango covered the pesticide without any treatment		Original	2.84 ± 0.2	
Mango fruits were only soaked in distilled water for 15 min		Control	1.59^{bc} ± 0.4	44.27^{de} ± 13.2
2	1	2L_1min	0.96^{ef} ± 0.02	66.41^{ab} ± 0.9
2	5	2L_5min	1.07^{c-f} ± 0.1	62.30^{a-d} ± 4.4
2	10	2L_10min	0.91^{ef} ± 0.2	68.25^{ab} ± 6.2
2	15	2L_15min	1.75^{ab} ± 0.4	38.33^{ef} ± 13.5
5	1	5L_1min	1.73^{ab} ± 0.03	38.98^{ef} ± 1.1
5	5	5L_5min	0.74^{f} ± 0.01	74.02^{a} ± 0.3
5	10	5L_10min	0.85^{ef} ± 0.01	70.16^{ab} ± 0.2
5	15	5L_15min	1.36^{b-e} ± 0.3	52.35^{b-e} ± 8.6
8	1	8L_1min	1.53^{b-d} ± 0.1	45.99^{c-e} ± 3.4
8	5	8L_5min	0.78^{f} ± 0.2	72.58^{a} ± 6.2
8	10	8L_10min	1.02^{d-f} ± 0.3	64.02^{a-c} ± 9.2
8	15	8L_15min	2.16^{a} ± 0.4	24.07^{f} ± 3.9

All the data are expressed as mean ± SD. Means in column under the same observation with different letters are significantly different ($p \leq 0.05$).

4.1.2 Cypermethrin

Table 4.2 displays the concentration and reduction rate of cypermethrin residues covered on mango surface under different flow rates of Ar gas and NTP treatment time.

It can be noticed that the remaining fraction of cypermethrin undulated with the plasma treatment time varying from 1 to 15 min. The removal efficacy of this pesticide

was significantly ($p \leq 0.05$) higher with NTP treatments conducted at 5 min and 15 min than at 1 min and 10 min, regardless of the Ar gas flux. The control which was mango covered cypermethrin and soaked in distilled water for 15 min gave 50.96 % of reduction rate from the original one (7.02 ppm), while concentration of the pesticide residues in NTP treated samples decreased by more than 60 % from the same initial level after 5 and 15 min of plasma operation at all of Ar flow rate.

Table 4.2 Effect of NTP treatments on degradation of cypermethrin residues in mango

Flow rate of Ar gas (L/min)	NTP treatment time (min)	Treatment	Concentration (ppm)	Reduction rate (%)
Mango covered the pesticide without any treatment		Original	7.02 ± 0.6	
Mango fruits were only soaked in distilled water for 15 min		Control	$3.44^{bc} \pm 0.3$	$50.96^{de} \pm 4.6$
2	1	2L_1min	$4.88^{a} \pm 1.1$	$30.42^{f} \pm 14.9$
2	5	2L_5min	$2.13^{d-f} \pm 0.1$	$69.61^{a-c} \pm 0.9$
2	10	2L_10min	$2.89^{c-e} \pm 0.5$	$58.71^{b-d} \pm 7.1$
2	15	2L_15min	$1.71^{ef} \pm 0.1$	$75.64^{ab} \pm 2.0$
5	1	5L_1min	$4.65^{a} \pm 0.04$	$33.68^{f} \pm 0.5$
5	5	5L_5min	$2.76^{c-f} \pm 0.4$	$60.72^{a-d} \pm 6.3$
5	10	5L_10min	$4.63^{a} \pm 0.6$	$34.04^{f} \pm 8.1$
5	15	5L_15min	$2.24^{c-f} \pm 0.3$	$68.06^{a-d} \pm 3.9$
8	1	8L_1min	$4.49^{ab} \pm 0.2$	$35.91^{ef} \pm 2.6$
8	5	8L_5min	$2.56^{c-f} \pm 0.1$	$63.45^{a-d} \pm 1.9$
8	10	8L_10min	$2.98^{cd} \pm 0.9$	$57.47^{cd} \pm 13.3$
8	15	8L_15min	$1.58^{f} \pm 0.6$	$77.43^{a} \pm 8.7$

All the data are expressed as mean ± SD. Means in column under the same observation with different letters are significantly different ($p \leq 0.05$).

According to Codex Alimentarius Commission, FAO/WHO Food Standard Programme (2016), MRLs of cypermethrin on mango is 0.7 ppm. Although none of

control and NTP treatments could reduce the pesticide concentration from the initial amount of 7.02 ± 0.6 pm to under the level of Codex MRLs, a large proportion of cypermethrin (> 60 %) removed by applying this cold plasma technique provides the high potential alternative approach to decrease and decompose cypermethrin contaminant not only in mango, but in other fresh produce also. In research of Pakvilai *et al*. (2011) conducted in four intensive agricultural areas of the Fang district, Chiang Mai, northern Thailand, the highest mean value of cypermethrin in fruits was at about 0.854 ppm. If the fruits are treated with the above selected NTP conditions possessing decontamination efficacy more than 60 %, the safe level of cypermethrin which is lower than 0.7 ppm can be obtained within very short treatment duration.

4.1.3 Carbendazim

The original concentration of carbendazim residues on mango after immersing in the pesticide solution was analysed to be 2.5 ± 0.09 ppm. Table 4.3 illustrates the residual concentration and percentage reduction of carbendazim on mango surface after cold plasma treatments as well as those of the control which was covered with the same carbendazim level and dipped in only distilled water for 15 min. It can be observed that the pesticide degradation efficiency increased significantly ($p \leq 0.05$) when the treatment time prolongs at Ar gas flow rate of 2 L/min and 8 L/min, while among NTP treatments operating with 5 L/min of Ar gas flux, the maximum reduction achieved for carbendazim was 74.34 % after treating 5 min. There was also significant ($p \leq 0.05$) difference in the reduction efficiency of carbendazim by applying NTP compared to the control. The results indicate that NTP treatments at several coded conditions such as 2L_10min, 2L_15min, 5L_5min or 8L_15 min gave the significantly ($p \leq 0.05$) higher impact on removing of carbendazim than the control.

It can be noticed that the remaining fraction of cypermethrin undulated with the plasma treatment time varying from 1 to 15 min. The removal efficacy of this pesticide was significantly ($p \leq 0.05$) higher with NTP treatments conducted at 5 min and 15 min than at 1 min and 10 min, regardless of the Ar gas flux. The control which was mango covered cypermethrin and soaked in distilled water for 15 min gave 50.96 % of reduction rate from the original one (7.02 ppm), while concentration of the pesticide

residues in NTP treated samples decreased by more than 60 % from the same initial level after 5 and 15 min of plasma operation at all of Ar flow rate.

Table 4.3 Effect of NTP treatments on degradation of carbendazim residues in mango

Flow rate of Ar gas (L/min)	NTP treatment time (min)	Treatment	Concentration (ppm)	Reduction rate (%)
Mango covered the pesticide without any treatment		Original	2.5 ± 0.09	
Mango fruits were only soaked in distilled water for 15 min		Control	0.98^{c-e} ± 0.1	60.84^{b-d} ± 4.3
2	1	2L_1min	1.51^{ab} ± 0.2	39.90^{ef} ± 8.5
2	5	2L_5min	1.70^{a} ± 0.1	32.31^{f} ± 2.9
2	10	2L_10min	0.70^{ef} ± 0.1	72.10^{ab} ± 5.7
2	15	2L_15min	0.79^{d-f} ± 0.1	68.29^{a-c} ± 5.0
5	1	5L_1min	1.02^{c-e} ± 0.1	59.47^{b-d} ± 3.0
5	5	5L_5min	0.65^{ef} ± 0.02	74.34^{ab} ± 0.8
5	10	5L_10min	1.10^{cd} ± 0.03	56.15^{cd} ± 1.2
5	15	5L_15min	1.32^{bc} ± 0.3	47.41^{de} ± 10.3
8	1	8L_1min	1.32^{bc} ± 0.2	47.34^{de} ± 7.4
8	5	8L_5min	1.11^{cd} ± 0.3	55.71^{cd} ± 13.3
8	10	8L_10min	0.96^{c-f} ± 0.04	61.85^{a-d} ± 1.3
8	15	8L_15min	0.60^{f} ± 0.01	75.96^{a} ± 0.7

All the data are expressed as mean ± SD. Means in column under the same observation with different letters are significantly different ($p \leq 0.05$).

The MRLs of carbendazim on berries and other small fruits is 1 ppm, while MRLs of this pesticide on mango is 5 ppm according to Codex Alimentarius Commission, FAO/WHO Food Standard Programme (2016). In the research about pesticide in food and plant derived from Southeast Asia, the fungicides carbendazim was observed in 66 samples (9.2 %) and a total of these samples had findings that were

above the MRLs (Skretteberg *et al*., 2015). Although applied immersion method for covering the pesticide did not introduce the residue concentration onto mangoes over the Codex MRLs, high degradation degrees of carbendazim by applying selected NTP conditions for a short period of treatment time demonstrated the high promising potential of cold plasma for decontamination of this pesticide residue in a wide range of fresh agricultural products.

Generally, from the whole obtained results about effect of NTP generated by GA discharge on decontamination of pesticide residues including chlorpyrifos, cypermethrin and carbendazim covered on mango surface, cold plasma treatment coded 5L_5min offered the significantly ($p \leq 0.05$) high reduction rate for all three types of pesticide with moderate levels of operating parameters which were 5 L/min of Ar flow rate and 5 min of treatment time.

Other researches have illustrated that it is possible to reduce or remove a number of pesticides residues by applying NTP. For instance, the degradation of paraoxon and parathion covered on glass slide with an atmospheric radio-frequency (RF) plasma in Ar/O_2 mixture was also reported by Kim *et al*. (2007). The key species responsible for oxidation of parathion and paraoxon were suggested to be the reactive species generated by plasma such as atomic oxygen, $OH^{\bullet}$ radical and molecule of nitrogen (Kim *et al*., 2007). Similarly, Bai *et al*. (2009) investigated the reduction of dichlorvos (DDV) and omethoate sprayed onto maize samples when applying an inductively coupled plasma (ICP) RF source operating in oxygen. At 120 W of discharge power, 120 s of treatment time, and 40 cm^3/min of O_2 flux, a complete degradation of the pesticides was found. The decontamination pathway was determined by applying a radical scavenger (t-butanol) and the most of identified intermediates were confirmed to be much less toxic than the original forms of pesticides (Bai *et al*., 2009). Bai *et al*. (2010) also observed the dissipation of DDV pesticides cover on solid phase using the same ICP source. However, the obtained results showed that the increase in applied power of plasma treatment gave an insignificant effect on the removal of DDV. The decomposition of malathion covered a filter paper when exposed to an atmospheric pressure plasma jet operating in He/O_2 has been demonstrated as well. The double bond P=S of malathion is oxidized to P=O by the energetic oxygen species of plasma resulting in formation of diethyl 2-(dimethoxyphosphorylthio) maleate (malaoxon) and

degradation was continued from attack at P-S and S-C bonding (Zhu *et al*., 2010). Misra *et al*. (2014b) using in-package gas phase plasma treatment of strawberries covered with azoxystrobin, cyprodinil, fludioxonil and pyriproxyfen reported similar results. In detail, the concentration of azoxystrobin, cyprodinil, fludioxonil and pyriproxyfen decreased by 69 %, 45 %, 71 % and 46 %, respectively, after 5 min of treatment at 80 kV. The degradation kinetics demonstrated that the increase in time treatment led to the rate of pesticide dissipation decreased. The end product of degradation pathway of fludioxonil pesticide identified by mass spectrometry was a carboxylic acid possessing low toxicity and health risk (Misra *et al*., 2014b). In research of Heo *et al*. (2014), fresh apples were coated with paraoxon and exposed to DBD plasma with pure air in a commercial refrigerator. The average of paraoxon concentration was decreased by 95.9 %.

In all investigations, it can be seen clearly that optimization of NTP effects on decontamination of pesticides depended on the process variables including treatment time, applying power, frequency, electrode contact area, discharge gap, distance from plasma source, type of discharge gas, pressure and flow rate of gas together with the packaging of material (Misra *et al*., 2014b; Pankaj *et al*., 2014).

4.1.4 Optical emission spectroscopy (OES) and concentration of H_2O_2 generated by gliding arc discharge NTP

The energy fraction derived from the NTP causing excitement of plasma chemical species to higher states of energy, which can be detected by the emission spectra. By applying OES, the emitted radiation spectrum of NTP generated by GA discharge was recorded and the intensity was quantified as function of wavelength. Figure 4.1 shows the emission spectrum of the plasma GA discharge at 5 L/min of Ar gas after 1 min and 200-800 nm of wavelength.

The emission spectrum clearly demonstrated the emission in the near UV region (200-400 nm). The OH peak around 310 nm was noticeable with relatively highly intense emission. The area 750 and 780 nm attributed to O atoms was also observed. These results demonstrated that the NTP generated by GA discharge was a source of reactive oxygen species (ROS).

The emission signal of hydroxyl radicals (OH•) which plays the key role in the decontamination of pesticide residues due to the high oxidation potential (E^0=2.8 V_{NHE}) (Bai *et al*., 2010; Hu *et al*., 2013; Jiang *et al*., 2014) were plotted in Figure 4.2 following to the applied NTP conditions for decontamination pesticide residues in mango.

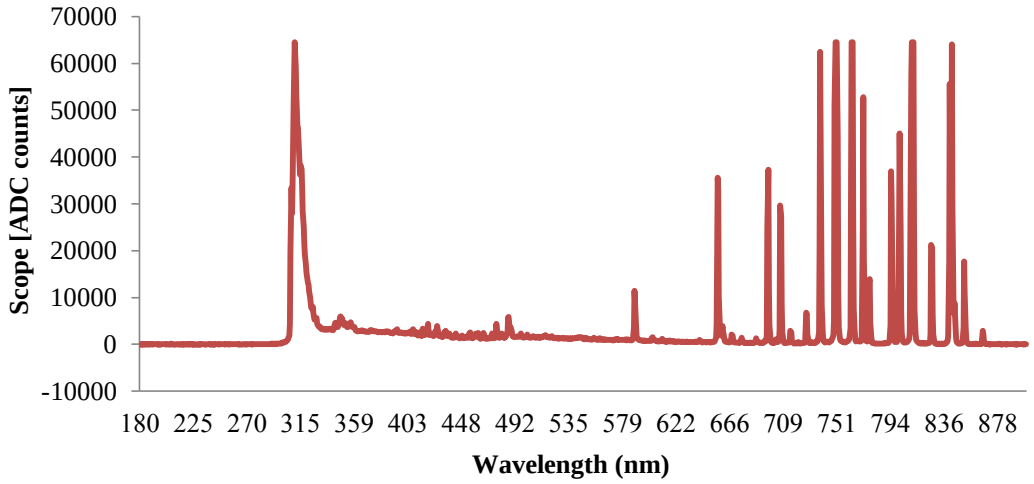


Figure 4.1 Optical emission spectrum of NTP generated by GA discharge

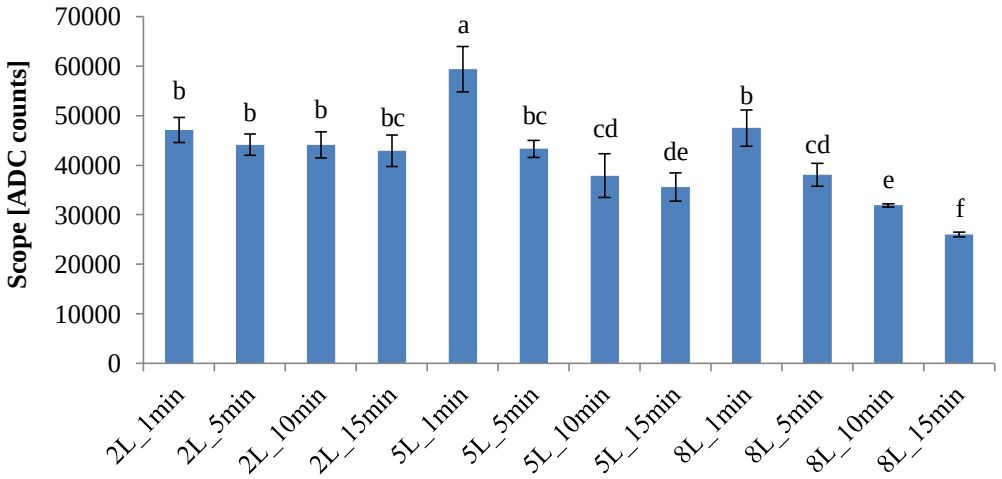


Figure 4.2 Emission signal at 310 nm of wavelength (OH radicals spectrum) of different NTP treatments for decontamination of pesticide residues

Means values with different letters are significantly different ($p \leq 0.05$). 2L, 5L and 8L indicate the flow rate of Ar gas per min, while 1-15 min represent the NTP treatment time.

According to Figure 4.2, Ar gas flux of 5 L/min offered the highest signal of the OH• active radicals after 1 min. This can be explained that the higher flow rate of Ar

gas would help to transfer more vapor of micro-bubble water through the GA discharge to generate plasma, however, when the flow rate was increased more, which did not allow water to be exposed for long time enough to the GA discharge and the vapor of micro-bubble water would move out of plasma system more quickly instead of contacting with GA discharge to produce active species like $OH^{\bullet}$ radicals. In addition, even though the significantly ($p \leq 0.05$) higher signal of $OH^{\bullet}$ radicals observed at the shorter treatment period of NTP rather than the longer one for all levels of Ar gas flux, the optimum combination between the high amount of $OH^{\bullet}$ radicals and the suitable duration of time for the interaction of plasma energy, reactive species and pesticide molecules is also the critical factor which mostly decides the efficacy of pesticide degradation.

The direct exposure of water vapor to GA discharge could also effectively produce hydrogen peroxide (H_2O_2), another reactive agents possessing high oxygen potential (E^0=1.77 V_{NHE}) (Hu *et al*., 2013), as illustrated in Figure 4.3.

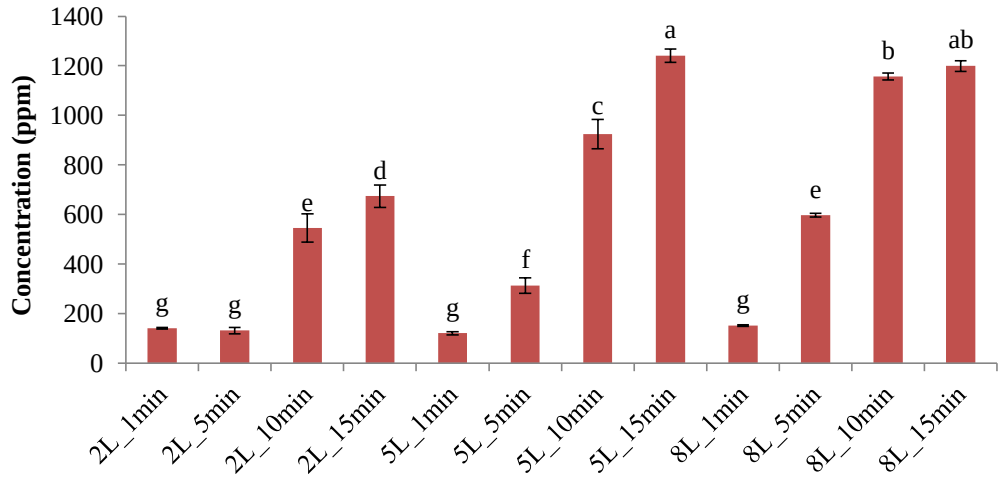


Figure 4.3 H_2O_2 concentration according to different NTP conditions for decontamination of pesticide residues

Means values with different letters are significantly different ($p \leq 0.05$). 2L, 5L and 8L indicate the flow rate of Ar gas per min, while 1-15 min represent the NTP treatment time.

The presence of H_2O_2 is a reasonable indicator for the formation of hydroxyl radicals ($OH^{\bullet}$) by plasma discharge with water (Joshi *et al*., 1995; Burlica *et al*., 2010) and from the analyzed results shown in Figure 4.3, the concentration of H_2O_2 in water samples was significantly ($p \leq 0.05$) higher in amount at the longer NTP treatment time

and the faster Ar gas flow rate. This can be explained by the quite longer half-life of H_2O_2 in plasma treated samples comparing to other active species (Kim *et al*., 2013). Accordingly, treated samples at NTP conditions including flow rate of plasma forming gas and treatment duration which possessed highly in both $OH^{\bullet}$ radicals and H_2O_2 concentration achieved the significantly better results in pesticide (chlorpyrifos, cypermethrin and carbendazim) decontamination capability as discussion in above sections.

4.1.5 Physicochemical properties of mango before and after NTP treatment

The effect of cold plasma treatments on quality indexes of mango fruits including surface color, texture, moisture content, total soluble solid (TSS), titratable acidity (TA), contents of ascorbic acid, carotenoid and total phenolic compounds were evaluated and compared with raw material (the control) without any NTP treatment.

4.1.5.1 Physical quality indexes

4.1.5.1.1 Mango color

Color is one of the most important quality attributes for the consumer's acceptability of fresh fruits (Misra *et al*., 2014c; Ma *et al*., 2015b). The values of color parameters of mango treated plasma and the control are presented in Table 4.4. There were significantly different ($p \leq 0.05$) among L*, a* and b* values of the control and NTP treated mangos. These variations may be caused by the ripe stage of mango which might have been slight affected by NTP treatments because color data correspond to the stage of maturity showing that the fruits were ripe with an increase in carotenoids synthesis (Sellamuthu *et al*., 2013). UV radiation produced by NTP can also make the impact on the color alteration of fresh produce such as apple (Falguera *et al*., 2011), watermelon (Feng *et al*., 2013), tomato (Bhat, 2016), and even Nam Dok Mai mango (Safitri *et al*., 2015). However, overall mango color change (ΔE*) was not significantly different ($p>0.05$) among treatments. Alterations in color parameters of corn salad leaves (Baier *et al*., 2014) and bell peppers (Vleugels *et al*., 2005) which were treated NTP have been observed. However, color of strawberries and tomatoes applied NTP shows insignificant changes (Misra *et al*., 2014c; Bermúdez-Aguirre *et al*., 2013).

Table 4.4 Color parameters of the control and mango treated NTP

Test ID	L*	a*	b*	ΔE*[ns]
Control	$72.66^{a-c} \pm 1.2$	$-6.69^{a-c} \pm 0.7$	$34.61^{c} \pm 2.4$	
2L_1min	$72.19^{b-d} \pm 2.4$	$-7.39^{b-d} \pm 1.3$	$36.53^{a-c} \pm 1.9$	4.39 ± 1.1
2L_5min	$72.90^{ab} \pm 1.6$	$-6.58^{a-c} \pm 1.6$	$35.89^{bc} \pm 1.6$	3.49 ± 1.7
2L_10min	$73.38^{ab} \pm 0.7$	$-7.74^{cd} \pm 1.8$	$35.53^{bc} \pm 2.6$	4.61 ± 1.8
2L_15min	$72.31^{b-d} \pm 1.1$	$-8.37^{d} \pm 1.0$	$35.84^{bc} \pm 2.9$	3.56 ± 1.4
5L_1min	$70.99^{d-f} \pm 1.5$	$-6.75^{a-c} \pm 1.5$	$38.10^{a} \pm 1.0$	4.81 ± 0.8
5L_5min	$72.95^{ab} \pm 1.0$	$-5.88^{a} \pm 0.8$	$37.49^{ab} \pm 1.1$	3.82 ± 1.8
5L_10min	$71.41^{c-f} \pm 2.1$	$-6.51^{a-c} \pm 0.7$	$37.11^{ab} \pm 1.9$	3.72 ± 0.8
5L_15min	$73.38^{bc} \pm 0.9$	$-6.27^{ab} \pm 1.1$	$36.33^{a-c} \pm 1.0$	3.71 ± 1.4
8L_1min	$72.01^{b-e} \pm 1.3$	$-7.26^{b-d} \pm 1.1$	$36.91^{ab} \pm 1.5$	3.03 ± 1.5
8L_5min	$70.61^{ef} \pm 1.4$	$-7.35^{b-d} \pm 0.6$	$35.64^{bc} \pm 1.5$	3.35 ± 1.4
8L_10min	$74.13^{a} \pm 0.9$	$-6.53^{a-c} \pm 1.3$	$35.55^{bc} \pm 2.4$	3.12 ± 1.5
8L_15min	$70.30^{f} \pm 1.1$	$-6.87^{a-c} \pm 1.1$	$36.05^{bc} \pm 1.5$	3.54 ± 1.4

[ns] indicates no significantly different ($p>0.05$)

All the data are expressed as mean ± SD. Mean in column under the same observation with different letters are significantly different ($p \leq 0.05$).

2L, 5L and 8L indicate the flow rate of Ar gas per min, while 1-15 min represent the NTP treatment time.

4.1.5.1.2 Mango texture

Texture parameters including hardness and springiness of the control and samples treated NTP were not significantly different ($p>0.05$) (Table 4.5). There was also an insignificant difference in the firmness values of treated cold plasma tomatoes when comparing with the control (Misra *et al*., 2014a). Ma *et al*. (2015b; 2016) found similarly that plasma activated water (PAW) could maintain the firmness of strawberries and bayberries. The cohesiveness, gumminess and chewiness showed some significant ($p \leq 0.05$) variations among treatments and treatments with control but

in not very large range of values. It might be said that the NTP treatments would not affect a lot on texture quality of mango fruits.

Table 4.5 Texture profile analysis (TPA) of the control and mango treated NTP

Test ID	Hardness^{ns} (N)	Cohesiveness (unitless)	Springiness^{ns} (mm)	Gumminess (N)	Chewiness (N.mm)
Control	3.27 ± 0.6	0.16^{a-c} ± 0.02	0.42 ± 0.08	0.51^{a-d} ± 0.08	0.22^{ab} ± 0.06
2L_1min	3.29 ± 0.6	0.14^{c} ± 0.01	0.48 ± 0.07	0.46^{cd} ± 0.06	0.22^{ab} ± 0.03
2L_5min	3.28 ± 0.9	0.15^{bc} ± 0.02	0.51 ± 0.08	0.49^{a-d} ± 0.15	0.25^{ab} ± 0.07
2L_10min	3.79 ± 0.8	0.16^{a-c} ± 0.04	0.47 ± 0.07	0.58^{ab} ± 0.12	0.27^{a} ± 0.07
2L_15min	3.69 ± 0.9	0.17^{a-c} ± 0.04	0.46 ± 0.11	0.59^{a} ± 0.11	0.28^{a} ± 0.10
5L_1min	3.45 ± 0.9	0.15^{bc} ± 0.02	0.44 ± 0.08	0.51^{a-d} ± 0.08	0.22^{ab} ± 0.06
5L_5min	3.32 ± 0.3	0.15^{bc} ± 0.01	0.52 ± 0.11	0.49^{b-d} ± 0.06	0.25^{ab} ± 0.05
5L_10min	3.01 ± 0.6	0.18^{a} ± 0.02	0.52 ± 0.05	0.54^{a-c} ± 0.11	0.28^{a} ± 0.06
5L_15min	2.99 ± 0.4	0.15^{bc} ± 0.02	0.46 ± 0.04	0.43^{d} ± 0.04	0.20^{b} ± 0.02
8L_1min	3.47 ± 0.6	0.14^{bc} ± 0.01	0.47 ± 0.08	0.50^{a-d} ± 0.10	0.23^{ab} ± 0.04
8L_5min	3.33 ± 0.4	0.16^{a-c} ± 0.02	0.49 ± 0.05	0.54^{a-c} ± 0.06	0.27^{a} ± 0.05
8L_10min	3.37 ± 0.5	0.15^{bc} ± 0.03	0.49 ± 0.05	0.49^{a-d} ± 0.04	0.24^{ab} ± 0.03
8L_15min	2.98 ± 0.4	0.17^{ab} ± 0.02	0.52 ± 0.05	0.51^{a-d} ± 0.08	0.26^{a} ± 0.06

^{ns} indicates no significantly different ($p>0.05$)

All the data are expressed as mean ± SD. Means in column under the same observation with different letters are significantly different ($p\leq0.05$).

2L, 5L and 8L indicate the flow rate of Ar gas per min, while 1-15 min represent the NTP treatment time.

4.1.5.1.3 Moisture and TSS content of mango

The results of moisture and TSS content of the control and NTP treated mangoes are displayed in Figure 4.4 and 4.5, respectively. There was non-significant difference in moisture content among NTP treated samples and control ($p>0.05$). However, there were significantly ($p\leq0.05$) higher TSS content of NTP

treatments coded 2L_5min, 5L_5min, 5L_10min, 8L_1min and 8L_5min ($p \leq 0.05$) than the control.

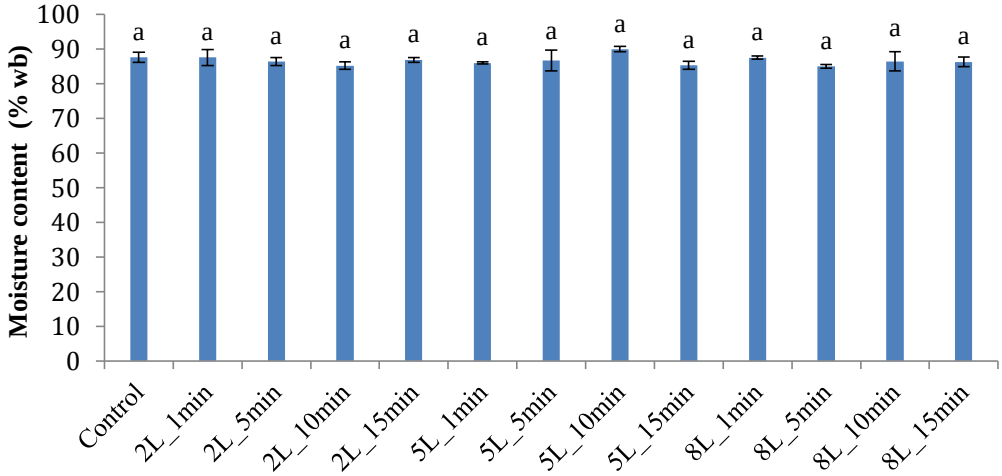


Figure 4.4 Moisture content of the control and mango treated NTP

2L, 5L and 8L indicate the flow rate of Ar gas per min, while 1-15 min represent the NTP treatment time.

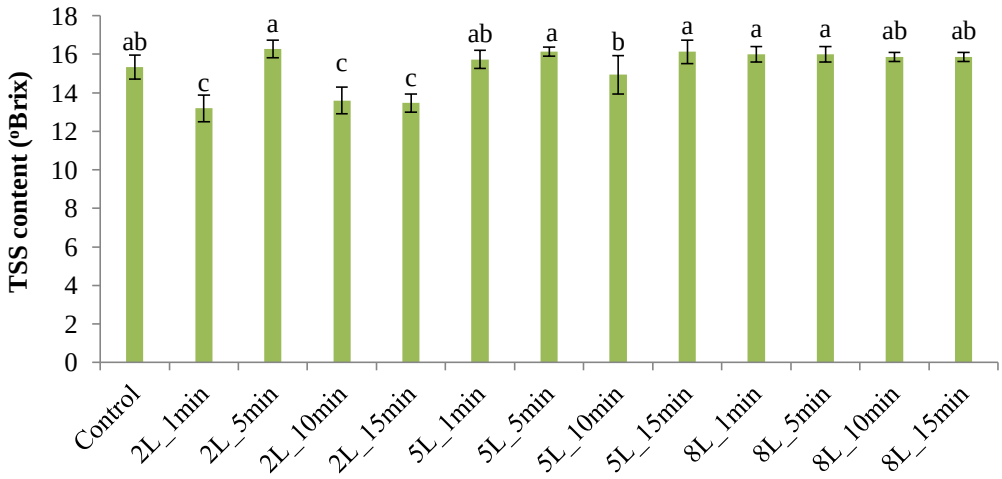


Figure 4.5 Total soluble solid (TSS) content of the control and mango treated NTP

Means values with different letters are significantly different ($p \leq 0.05$). 2L, 5L and 8L indicate the flow rate of Ar gas per min, while 1-15 min represent the NTP treatment time.

As the main substrates of respiratory metabolism, sugars and acids are consumed, causing corresponding changes in TSS of fruits (Ma *et al*., 2016). Higher contents of TSS in plasma treated mangoes might be due to the effect of NTP treatments on the respiratory rate of mango which consequently increased the consumption of sugars and acids of this fruit. A significant increase in TSS values of

blueberries treated atmospheric cold plasma was also observed by Sarangapani *et al*. (2017).

4.1.5.1.4 TA values of mango

TA is an essential parameter used to evaluate storage characteristics of fruits. High decrease in value of TA reflects the senescence of fruits (Khaliq *et al*., 2015). In this study, TA percentage of mangoes decreased significantly ($p \leq 0.05$) with all NTP treatment durations of 5 L/min and 8 L/min of Ar flux when comparing with this of the controlled sample (Figure 4.6). Titratable acidity decrease with advancing fruit ripening (Khaliq *et al*., 2015) and NTP treatment at high flow rate of Ar gas may cause some effects supporting the faster maturity of mango. Moreover, the reaction of fruit's cells with reactive oxygen species (ROS) generated by plasma chemistry could be used to explain the loss of fruit quality during processing (Misra *et al*., 2015).

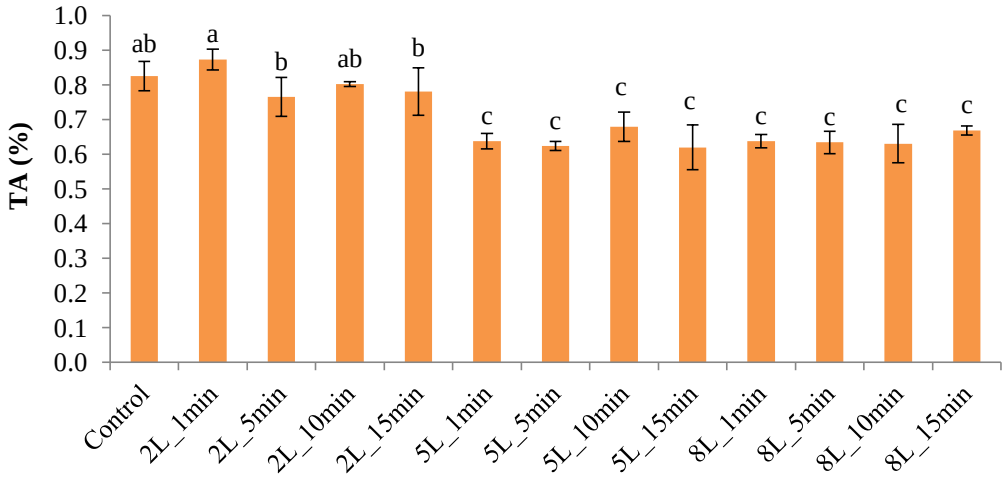


Figure 4.6 TA of mango flesh of control and plasma treatment

Means values with different letters are significantly different ($p \leq 0.05$). 2L, 5L and 8L indicate the flow rate of Ar gas per min, while 1-15 min represent the NTP treatment time.

4.1.5.2 Chemical quality indexes

4.1.5.2.1 Ascorbic acid content of mango

Ascorbic acid is water soluble and a powerful antioxidant acting to prevent or reduce the damage caused by ROS in fruits. Fruits are a natural source of ascorbic acid and losses occur during ripening (Khaliq *et al*., 2015). In mango flesh,

ascorbic acid content ranges from 9.79 to 186 mg/100g FW (Ribeiro and Schieber, 2010). As indicated in Figure 4.7, ascorbic acid content of the fruit declined significantly ($p \leq 0.05$) with all NTP treatments comparing to the value of this in the control. In research about effects of NTP on chemical qualities of strawberries, Misra *et al*. (2015) also found that both applied voltage and treatment time of plasma caused a significant impact on the ascorbic content.

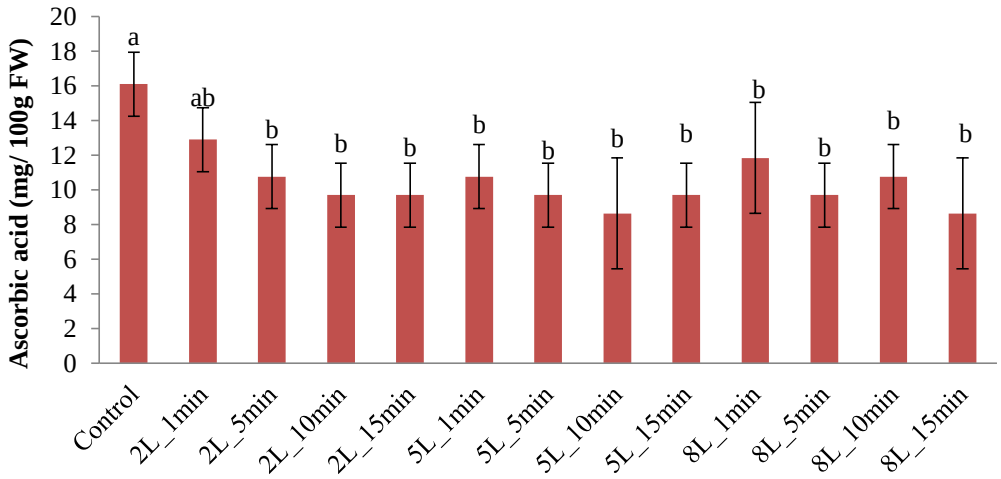


Figure 4.7 Ascorbic acid content of the control and mango treated NTP

Means values with different letters are significantly different ($p \leq 0.05$). 2L, 5L and 8L indicate the flow rate of Ar gas per min, while 1-15 min represent the NTP treatment time.

As the main reactive oxygen species (ROS) detoxifying compound scavenging hydroxyl radicals, superoxide anion and reducing hydrogen peroxide to water through the ascorbate peroxidase reaction (Blokhina *et al*., 2003; Khaliq *et al*., 2015), the reaction of ascorbic content of mango with ROS such as ozone, oxidizing species generated by NTP during processing might be used to explain for the loss of this compound observed after plasma treatments (Misra *et al*., 2015). There are also studies demonstrated the effect of UV radiation, one of the active components of NTP, on the decrease of ascorbic content in fresh-cut pineapples (Pan and Zu, 2012), apple juice (Falguera *et al*., 2011) and tomato juice (Bhat, 2016).

4.1.5.2.2 Carotenoid and total phenolic content of mango fruit

There is a wide variation in the total carotenoid content (TCC) of mango flesh ranged from 0.9 to 9.2 mg/100 g FW in respect of cultivars and maturity

(Ribeiro and Schieber, 2010; Sellamuthu *et al*., 2013). TCC of mango applied NTP was significantly ($p \leq 0.05$) higher for the longer treatment time of 5 and 8 min, regardless of Ar gas flow rate (Table 4.6). It is well known that carotenoids are responsible for the color and antioxidant properties in biological systems. Carotenoid synthesis in mangoes coincides with the ripening process and production of ethylene (Sellamuthu *et al*., 2013). The possible effect of NTP enhancing the maturity rate of mango might be used to explain the higher amount of TCC content in treated samples.

Table 4.6 Carotenoid and TPC of mango with and without NTP treatments

Test ID	Carotenoid (mg/100g FW)	TPC (mg GAE /100g FW)
Control	$1.98^{g} \pm 0.09$	$183.17^{a} \pm 4.5$
2L_1min	$2.03^{fg} \pm 0.06$	$170.38^{b} \pm 10.7$
2L_5min	$2.42^{e} \pm 0.12$	$153.55^{cd} \pm 3.8$
2L_10min	$2.46^{e} \pm 0.02$	$145.64^{de} \pm 8.0$
2L_15min	$2.56^{d} \pm 0.02$	$131.60^{f} \pm 5.2$
5L_1min	$1.94^{g} \pm 0.03$	$174.45^{ab} \pm 3.5$
5L_5min	$2.69^{bc} \pm 0.02$	$162.86^{bc} \pm 5.4$
5L_10min	$2.66^{cd} \pm 0.11$	$150.12^{d} \pm 11.9$
5L_15min	$3.34^{a} \pm 0.03$	$137.57^{ef} \pm 4.5$
8L_1min	$1.78^{h} \pm 0.05$	$153.69^{cd} \pm 5.6$
8L_5min	$2.13^{f} \pm 0.01$	$133.51^{ef} \pm 3.7$
8L_10min	$2.79^{b} \pm 0.01$	$136.66^{ef} \pm 9.8$
8L_15min	$2.73^{bc} \pm 0.05$	$136.67^{ef} \pm 0.5$

All data are expressed as mean ± SD. Means in column under the same observation with different letters are significantly different ($p<0.05$).

2L, 5L and 8L indicate the flow rate of Ar gas per min, while 1-15 min represent the NTP treatment time.

In contrast, the total phenolic content (TPC) showed the downward trend after NTP treatments (Table 4.6), the values of TPC decreased significantly ($p \leq 0.05$) with nearly all NTP conditions. Scavenging free radicals is known as the ability of phenolic

compound leading to cleavage of the central heterocyclic in polyphenolic skeleton as well as oligomerization subsequently (Elez Garofulić *et al*., 2015). Grzegorzewski *et al*. (2011) also observed the degradation of phenolic compounds in lamb's lettuce which was not made by photo- or thermo-desorption processes at the surface but by the combined interaction of various reactive species of plasma. The interactions with charged ions and reactive species like $OH^{\bullet}$, O and O_2 may cause an erosion of epidermal tissue layers of this vegetable from that flavonoids and other compounds stored in the central vacuoles of guard cells as well as epidermal cells are released and degraded (Grzegorzewski *et al*., 2011). Besides, effect of NTP on maturity of mango may be used to explain the decrease of TPC of the fruit treated cold plasma. During ripening, TPC of fresh fruits such as strawberries (Ornelas-Paz Jde *et al*., 2013), medlar fruit (Gruz *et al*., 2011) and mango (Ibarra-Garza *et al*., 2015; Siriamornpun and Kaewseejan, 2017) were observed to be reduced. In addition, effect of UV radiation on TPC of mango can be another possible reason for the drop in the bioactive compound. Safitri *et al*. (2015) reported that TPC of Nam Dok Mai mango was decreased after UV treatment compared to the control.

4.1.6 Sensory attributes of mango

The sensorial attributes of mango fruits treated with NTP (coded 5L_5min) which offered the high efficacy on degradation of three different types of pesticide were well accepted in overall acceptability by panelists (Table 4.7). All the tested sensory properties including color, aroma, flavor, texture and overall liking of mango were not different significantly ($p>0.05$) upon the cool plasma exposure as compared with untreated controls. In addition, sensory evaluation by panelists also found that mango fruits applied NTP were obtained significantly ($p\leq0.05$) higher scales for assessed color and flavor attributes than the samples with thermal treatment.

Mango is a high nutritious tropical fruit which is widely famous for its unique flavor and aroma (Kaushik *et al*., 2015). During ripening, starch of the fruit is converted into sugar because of the hydrolysis of starch granules in the chloroplast. According to observation of Sivakumar *et al*. (2011), sugar production from starch was decreased by hot water treatment (HWT) and carotenoid content of flesh fruit as well as texture properties were also affected during direct HWT. Furthermore, the adverse effect of

HWT on organolyptic attributes was observed when mature mango was treated with this technique (Sivakumar *et al*., 2011). There are deteriorative reactions including Maillard non-enzymatic browning, pigment damage together with flavonoid destruction caused during thermal treatment (Ahmed *et al*., 2002; Rawson *et al*., 2011; Kaushik *et al*., 2015), and they can be used to explain the alteration in color as well as sensitive flavor compounds leading to severe impact on organoleptic aspects of thermal treated mango flesh.

Table 4.7 Sensory attributes of NTP treated mango comparing with the control (raw fresh fruit) and conventional hot water treatment

Treatment	Color	Aroma^{ns}	Flavor	Texture^{ns}	Overall-liking^{ns}
Control	$7.20^{a} \pm 1.01$	7.00 ± 1.28	$7.00^{a} \pm 1.34$	6.88 ± 1.19	7.14 ± 1.01
NTP	$6.82^{ab} \pm 1.35$	6.74 ± 1.24	$6.76^{a} \pm 1.33$	6.82 ± 1.12	6.86 ± 1.07
Thermal	$6.34^{b} \pm 1.48$	6.44 ± 1.68	$6.04^{b} \pm 1.74$	6.68 ± 1.48	6.56 ± 1.49

^{ns} indicates no significantly different ($p>0.05$).

All data are expressed as mean ± SD. Means in column under the same observation with different letters are significantly different ($p\leq0.05$).

4.2 Effects of NTP on inactivation of *C. gloeosporioides* caused anthracnose disease in mango fruits

4.2.1 Effect of NTP on *C. gloeosporioides* mycelia growth

Following 8-day incubation at 28-33ºC, the growth of *C. gloeosporioides* mycelia on PDA petri dishes with and without NTP treatments are presented in Table 4.8. From the second day to the fifth day of observation, the expansion of mycelial colony treated with all NTP applied conditions was significantly ($p\leq0.05$) slower than the control. However, after 5 days of incubation, the effects of NTP on growth of mold were less and only 5L_7min gave the smaller expansion of fungal mycelium than the control significantly ($p\leq0.05$). For all NTP treatments and the control, the growth curves based on mycelia diameter were typical of a linear fungal growth for a period ranged from 1 to 8 days (Figure 4.8). Comparing with the control (k=9.3), the growth rate constant of *C. gloeosporioides* mycelium calculated as the slope of linear grow curve of 5L_7min NTP treatment (k=8.9) also showed the lower value. This indicates that the higher flow

rate of Ar gas and the longer treatment time are the major parameters of NTP which can enhance the inhibition efficiency of the mold. The higher flux of plasma forming gas might lead to the shorter time of transferring plasma active species like ROS, the key factors of antimicrobial efficacy, which normally have a quite short half-life, from the plasma generating region to the target surface, while the longer treatment time can also help to improve the interaction efficiency between fungus and reactive agents of plasma (Niemira and Sites, 2008). The effect of 5L_7min NTP treatment, which demonstrated the highest impact against the growth of *C. gloeosporioides* on PDA during 8-day incubation period, was illustrated more clearly by Figure 4.9.

According to Xu *et al*. (2017), cellular compounds may be destroyed by oxidative effect of ROS leading to disfunction of cell or cell death. ROS accumulation in spores and mycelium of the anthracnose mold induced by cool plasma treatments can be one of the mechanisms by which NTP suppressed the germination of spore as well as mycelium growth. In addition, respiratory activities of mitochondria which are the major source of cellular adenosine triphosphate (ATP) and play a central role in a variety of cellular processes are also the main endogenous sources of ROS, thus ROS accumulation can lead to mitochondrial degradation of fungal spore, oxidation of macromolecules, causing mtDNA mutations, aging and cell apoptosis, thereby inhibiting the development of anthracnose fungus.

Table 4.8 Mycelial growth of *C. gloeosporioides* on PDA petri dishes with and without NTP treatments

Test ID	Mycelial diameter (mm)							
	1st day	2nd day	3rd day	4th day	5th day	6th day	7th day	8th day
Control	5	19.13^{a} ± 0.6	29.69^{a} ± 0.2	41.40^{a} ± 0.6	49.29^{a} ± 0.7	56.59^{a} ± 1.3	65.09^{a} ± 1.8	70.94^{a} ± 1.6
1L_1min	5	14.82^{bc} ± 0.7	25.52^{bcd} ± 1.2	35.32^{b} ± 1.9	44.83^{b} ± 2.9	53.23^{ab} ± 2.0	61.00^{ab} ± 1.4	67.64^{ab} ± 1.0
1L_3min	5	14.86^{bc} ± 0.5	25.87^{cd} ± 0.9	35.50^{b} ± 1.2	44.83^{b} ± 0.5	53.53^{ab} ± 0.9	59.75^{b} ± 2.7	65.65^{b} ± 2.9
1L_5min	5	14.53^{bc} ± 0.8	25.02^{bcd} ± 1.0	35.63^{b} ± 0.5	44.67^{b} ± 1.9	53.65^{ab} ± 0.9	62.64^{ab} ± 1.8	68.67^{ab} ± 1.8
1L_7min	5	15.54^{bc} ± 1.3	26.49^{d} ± 1.5	36.92^{b} ± 2.6	45.57^{b} ± 3.1	54.52^{ab} ± 4.2	62.74^{ab} ± 3.6	68.52^{ab} ± 4.0
3L_1min	5	16.22^{b} ± 1.1	25.14^{bcd} ± 1.4	35.75^{b} ± 2.9	45.31^{b} ± 0.4	52.72^{b} ± 2.7	61.33^{ab} ± 3.2	67.21^{ab} ± 3.4
3L_3min	5	16.17^{b} ± 1.4	25.55^{bcd} ± 1.9	36.91^{b} ± 2.4	44.9^{b} ± 2.3	53.82^{ab} ± 2.1	61.35^{ab} ± 1.9	67.7^{ab} ± 1.7
3L_5min	5	16.10^{bc} ± 1.3	25.60^{bcd} ± 1.1	36.36^{b} ± 2.1	46.16^{b} ± 1.4	54.49^{ab} ± 1.2	62.92^{ab} ± 1.8	68.58^{ab} ± 1.7
3L_7min	5	15.62^{bc} ± 1.2	24.59^{bcd} ± 1.5	35.37^{b} ± 2.5	45.55^{b} ± 2.1	53.63^{ab} ± 2.3	62.14^{ab} ± 2.5	68.63^{ab} ± 3.5
5L_1min	5	15.12^{bc} ± 0.9	24.95^{bcd} ± 1.2	36.35^{b} ± 1.8	44.59^{b} ± 2.4	54.19^{ab} ± 2.5	62.96^{ab} ± 2.5	69.96^{ab} ± 2.3
5L_3min	5	14.18^{c} ± 1.1	23.84^{bc} ± 1.5	36.14^{b} ± 1.1	44.08^{b} ± 1.6	53.68^{ab} ± 1.3	62.04^{ab} ± 0.7	68.76^{ab} ± 1.1
5L_5min	5	15.00^{bc} ± 1.0	25.35^{bcd} ± 0.3	36.67^{b} ± 0.3	45.83^{b} ± 1.0	54.52^{ab} ± 1.5	62.02^{ab} ± 1.9	68.31^{ab} ± 2.5
5L_7min	5	14.00^{c} ± 1.9	23.72^{a} ± 1.7	35.16^{b} ± 1.4	43.87^{b} ± 1.6	52.45^{b} ± 1.9	59.87^{b} ± 4.0	65.39^{b} ± 4.9

All data are expressed as mean ± SD. Means in column under the same observation with different letters are significantly different ($p \leq 0.05$).

1L, 3L and 5L indicate the flow rate of Ar gas per min, while 1-7 min represent the NTP treatment time.

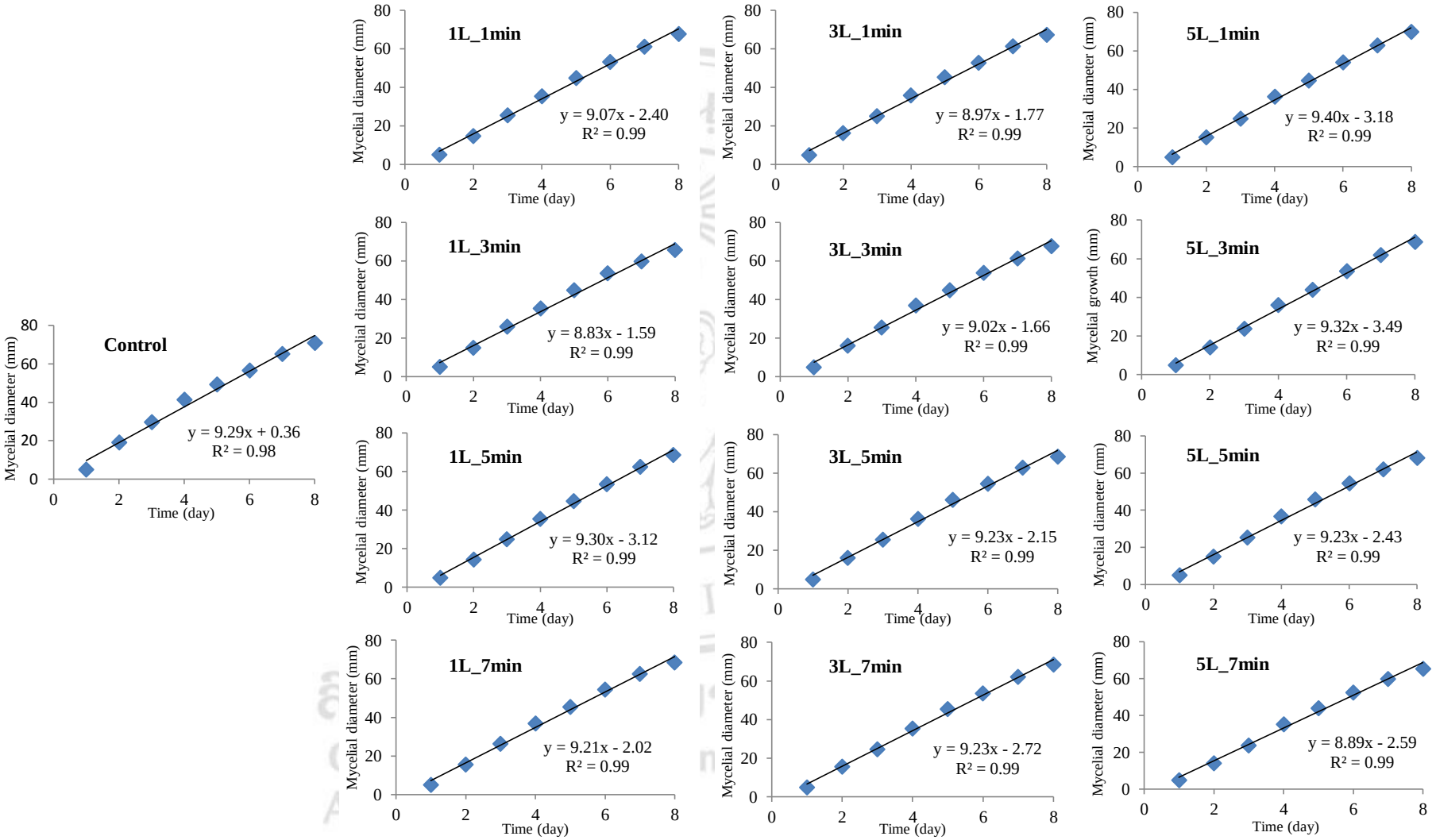


Figure 4.8 Effect of NTP treatments on mycelium growth of *C. gloeosporioides* during 8-day incubation period

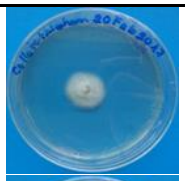
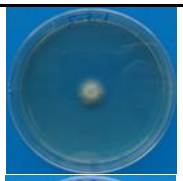
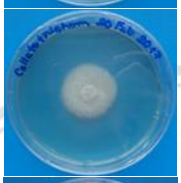
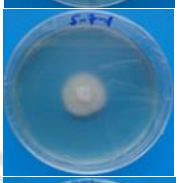
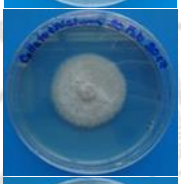
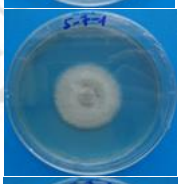
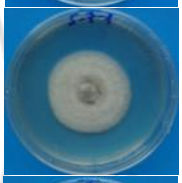
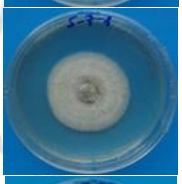
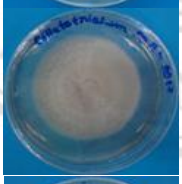
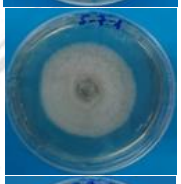
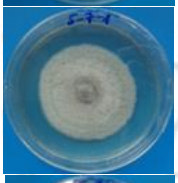
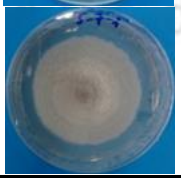
Time (days)	Control (non-plasma treatment)	5L_7min NTP treatment
1st day		
2nd day		
3rd day		
4th day		
5th day		
6th day		
7th day		
8th day		

Figure 4.9 Mycelial growth of *C. gloeosporioides* treated 5L_7min NTP (5 L/min of Ar flow rate and 7 min of treatment time) during 8-day incubation comparing with the control

4.2.2 Effects of NTP on *C. gloeosporioides* spores in suspension

The results from Figure 4.10 demonstrate that NTP treatments had the significantly ($p \leq 0.05$) impact on the number of *C. gloeosporioides* spores. The longer of treatment time and the higher of gas flow rate of Ar gas also gave the better results on reduction of *C. gloeosporioides* spores in solution. Among treatments, the maximum reduction of *C. gloeosporioides* spores was 1 log spore/mL after 7 min of NTP treatment with 5 L/min gas flux.

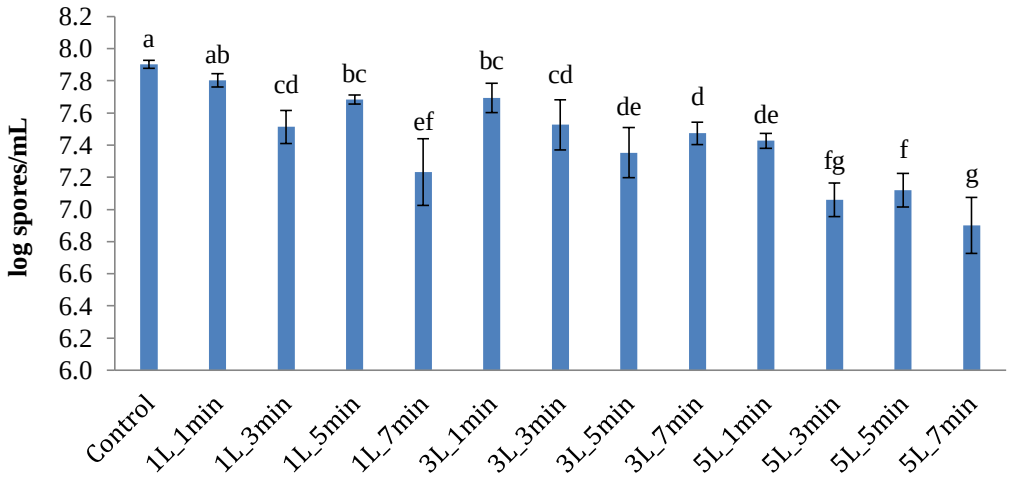


Figure 4.10 Effect of NTP treatments on number of *C. gloeosporioides* spore

Means values with different letters are significantly different ($p \leq 0.05$). 1L, 3L and 5L indicate the flow rate of Ar gas per min, while 1-7 min represent the NTP treatment time.

The inactivation potential towards microbial spores of NTP plasmas have been shown by many authors (Brandenburg *et al*., 2009; Moreau *et al*., 2008; Schnabel *et al*., 2012; van Gils *et al*., 2013) and the often described synergistic inactivation kinetics, for instance, of *Bacillus* spore strains are presumably due to combined inactivation effects of the NTP reactive species such as $O^{\bullet}$, O_2, O_3, $OH^{\bullet}$ and ultraviolet (UV) radiation since they are known to induce DNA strain breaks (Setlow, 2007) or to damage other proteins in the cell (Philip *et al*., 2002). Besides, UV can make the formation of DNA photoproducts including cyclobutane pyrimidine dimers and pyrimidine 6-4 pyrimidone, which have the high efficacies to inhibit transcription and replication and eventually lead to mutagenesis and cell death (Ma *et al*., 2017). However, there are also many researchers claim that UV emission plays a minor role in the inactivation of

microorganisms at atmospheric pressure, and the inactivation process is controlled by chemically reactive species (Knorr *et al*., 2011; Laroussi and Leipold, 2004). The intrinsic photodesorption and etching, which were attributed to explain the degradation and erosion of outer structures of microorganisms, leading to cell death are involved mechanisms (Reineke *et al*., 2015).

4.2.3 Effect of NTP on anthracnose disease severity of mango inoculated with *C. gloeosporioides*

In vivo test results about impact of NTP treatments on inhibition of anthracnose disease caused by *C. gloeosporioides* mold were presented in Figure 4.11, 4.12 and 4.13. The free of disease symptoms in mango fruit inoculated with only PDA medium (Figure 4.13) suggested the appropriateness of inoculation method for introducing the fungus onto mango fruits.

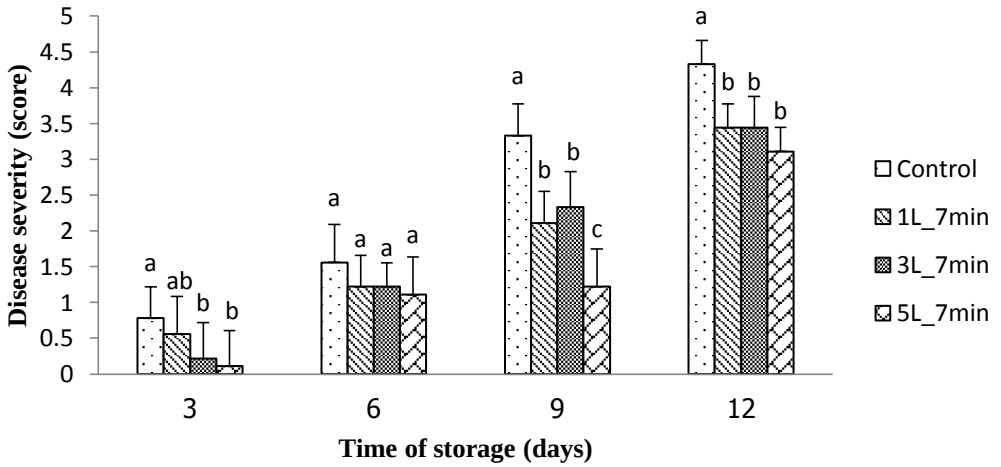


Figure 4.11 Severity of anthracnose disease of mango fruit treated with NTP conditions and the control during storage at 28-33ºC for 12 days

Means values with different letters are significantly different ($p \leq 0.05$). 1L, 3L and 5L indicate the flow rate of Ar gas per min, and 7 min represents the NTP treatment time.

The disease symptoms of anthracnose on artificially inoculated mango with and without NTP treatments appeared and developed during incubation time. However, there was a significant ($p \leq 0.05$) delay in disease development in the fruits treated NTP conditions coded 1L_7min, 3L_7min and 5L_7min during storage of 12 days at 28-33ºC, 95 % RH (Figure 4.11). The severity of disease in the controls increased sharply

and reached the highest scale (5=>40 % of disease symptoms on surface) after 12 days of storage (Figure 4.13). The highest fungistatic effect was obtained in inoculated fruits treated with NTP at 5 L/min Ar flow rate for 7 min, showing the lowest disease severity scale (3.11 ± 0.3) after 12 days of incubation.

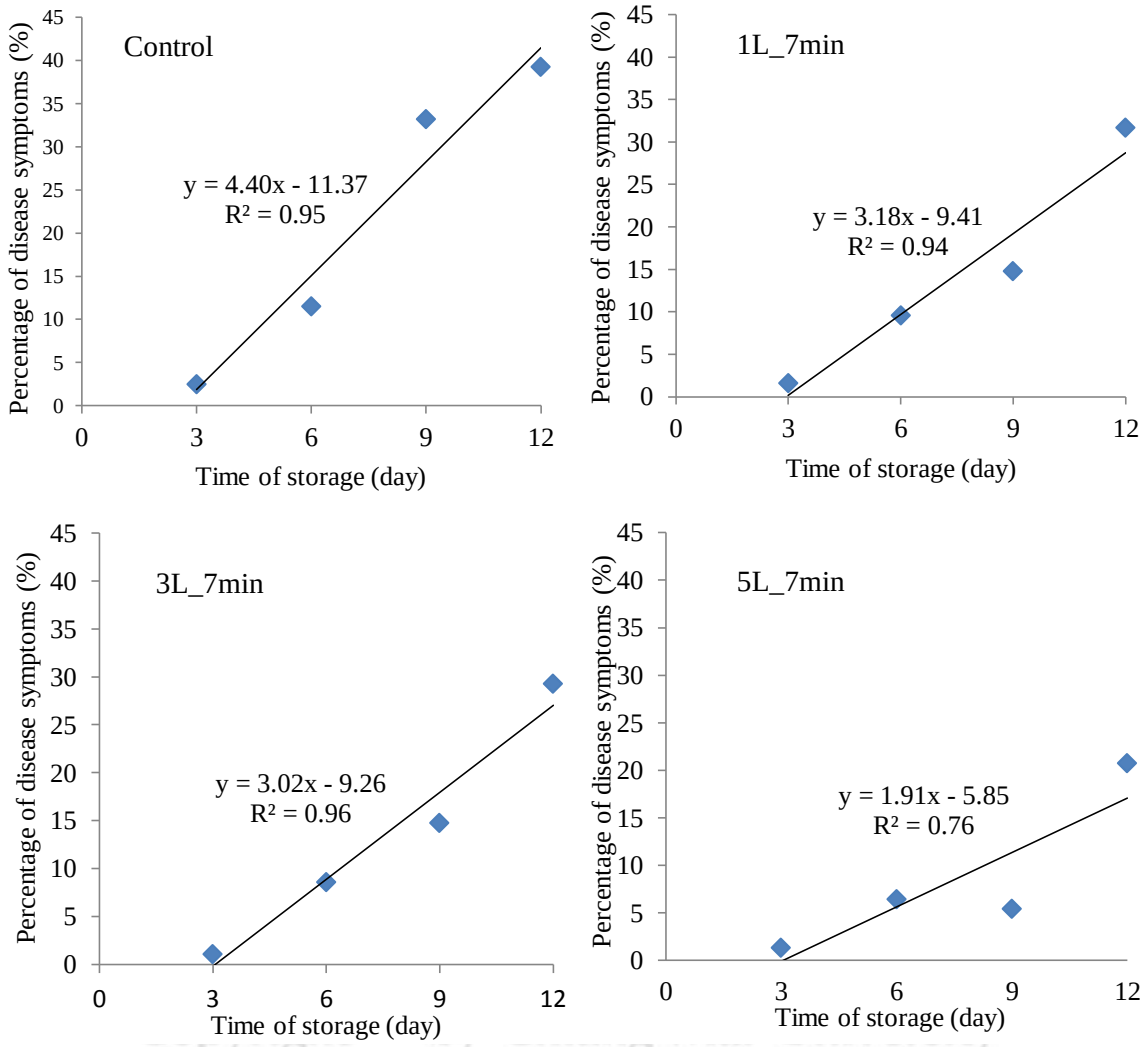


Figure 4.12 Percentage of disease symptoms on mango surface treated with NTP conditions and the control during storage at 28-33ºC for 12 days

To better observation the difference in growth of lesion caused by *C. gloeosporioides* among mango fruits with NTP treatments (1L_7min, 3L_7min and 5L_7min) and the control, the percentage of disease symptoms were plotted against the storage time (days) (Figure 4.12). The slope of each linear curve represented the growth rate of infected area. It can be seen clearly that all plasma treatments gave the lower rate

constant of percentage of disease symptoms than that of the control (k=4.4) and the lowest one (k=1.9) was obtained at 5L_7min NTP.

Time (days)	Control (non-plasma treatment)	5L_7min NTP treatment	Inoculated with PDA only
3rd day			
4th day			
5th day			
6th day			
7th day			
8th day			
9th day			
10th day			
11th day			
12th day			
13th day			
14th day			

Figure 4.13 Growth of anthracnose disease on inoculated mango fruits treated with 5L_7min NTP condition; untreated control and inoculated with only PDA during storage at 28-33°C for 14 days

The microbial inhibition effect of NTP plasma on disease severity of *C. gloeosporioides* might be attributed to the ROS generation in fungal spores resulting in mitochrondrial damage which may act as one of the involved mechanisms inhibiting spore growth of *C. gloeosporioides* and controlling anthracnose incidence in mango fruit (Xu *et al*., 2017). Through analysis of extractible proteins from the outer membrane of the microbe (pathogen of potato), Moreau *et al*. (2007) also found that GA plasma caused the release of microbial genomic DNA by inducing protein dimerization and aggregation supporting the antimicrobial effect of this technique based on oxidative mechanism.

4.2.4 Optical emission spectroscopy (OES) and concentration of H_2O_2 generated by GA discharge NTP

The emission signals of $OH^{\bullet}$ radicals detected by OES system and the quantity of H_2O_2 generated according to the applied plasma conditions to inactivate *C. gloeosporioides* mold causing anthracnose disease in mango fruits were presented in Figure 4.14 and 4.15, respectively.

In Figure 4.14, among three applied flow rate, 5 L/min of Ar gas flux offered the highest signal emission of the $OH^{\bullet}$ radicals. This can be explained that at the higher flow rate of gas, the more vapor of micro-bubble water were supported to transfer through the GA discharge to generate NTP. In addition, although the higher signal of $OH^{\bullet}$ radicals appeared at the shorter treatment period, then reduced in the longer one with all flux level of Ar gas, for instance 3L_1min or 5L_1min, the optimum combination between the high amount of generated $OH^{\bullet}$ radicals and suitable treatment time for the interaction of plasma energy, reactive species and microorganism is the key factor which mainly decides the NTP efficacy of microbial inactivation.

From the results displayed in Figure 4.15, the concentration of H_2O_2 formed in water samples with respect to NTP treatments for inhibition of *C. gloeosporioides* fungus showed the upward trend with the longer plasma treatment time and higher flow rate of Ar gas. Accordingly, 5L_7min NTP treatment gave the highest concentration of H_2O_2 as well as offering the best microbial inhibition capability among applied plasma treatments. Experimental obtained results indicate that GA discharge reacting with water under vapor form could effectively generate H_2O_2.

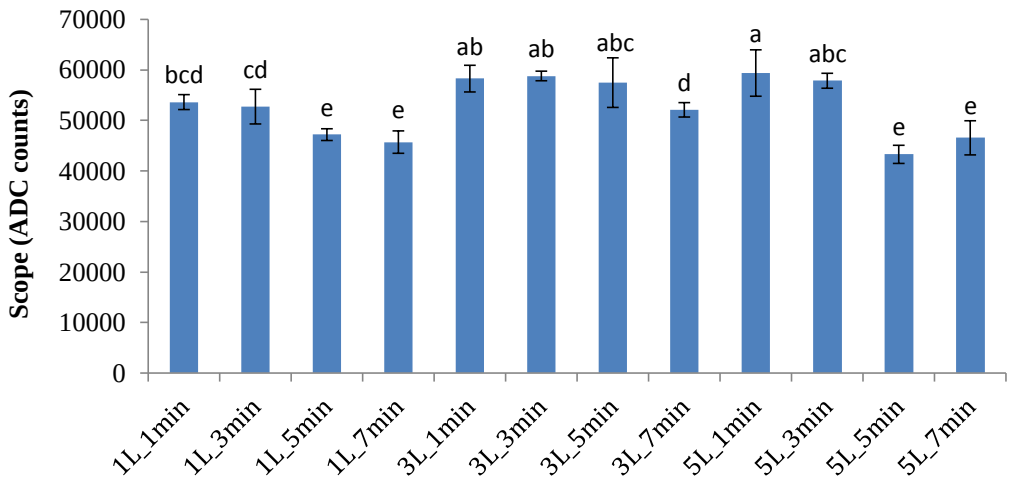


Figure 4.14 Emission signal at 310 nm of wavelength (OH radicals spectrum) of different NTP treatments for inhibition growth of *C. gloeosporioides*

Means values with different letters are significantly different ($p \leq 0.05$). 1L, 3L and 5L indicate the flow rate of Ar gas per min, while 1-7 min represent the NTP treatment time.

When exposing directly to GA discharge, the dissociation of water molecules can occur following to the reactions (Fridman, 2008; Parvulescu *et al*., 2012):

$$e + H_2O \rightarrow H^{\bullet} + {}^{\bullet}OH + e^-$$

$$e + H_2O \rightarrow H^- + {}^{\bullet}OH$$

$$M^+ + H_2O \rightarrow H_2O + M$$

$$H_2O^+ + H_2O \rightarrow H^+(H_2O) + OH^{\bullet}$$

Then, the recombination of hydroxyl radicals ($OH^{\bullet}$) can form H_2O_2 or in other words, the presence of H_2O_2 is a reasonable indicator for $OH^{\bullet}$ radicals formation by plasma discharge with water (Joshi *et al*., 1995; Burlica *et al*., 2010). In addition, it is known that H_2O_2 is one of the most chemically stable ROS having a direct antimicrobial impact and involved in the cross-linking of cell walls, signaling, induction of gene expression and hypersensitive cell death. Accumulation of H_2O_2 could inhibit biotrophic pathogens and played an important role in life style of *C. gloeosporioides* (Eloy *et al*., 2015).

There are a number of published papers exploring the effects on microbial inactivation by UV, ozone, radicals, super oxide and H_2O_2 produced from various types of plasma discharges (Basaran *et al*., 2008; Berardinelli *et al*., 2016; Dasan *et al*., 2016). However, antimicrobial efficacy of NTP is highly dependent not only on the plasma forming gas applied and the specification of the apparatus producing plasma which mainly determine the composition and density of plasma reactive species, but also on the intrinsic factors including water activity, texture, protein and fat content, pH and the type of foodstuff being treated (Lacombe *et al*., 2015; Lee *et al*., 2015).

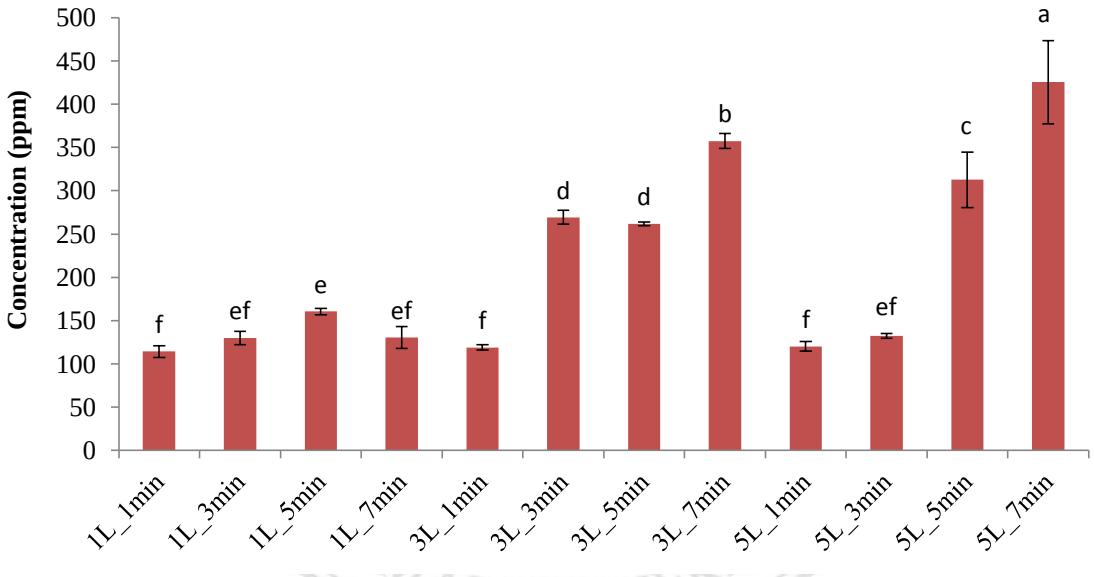


Figure 4.15 H_2O_2 concentration according to different NTP conditions for inhibition growth of *C. gloeosporioides*

Means values with different letters are significantly different ($p \leq 0.05$). 1L, 3L and 5L indicate the flow rate of Ar gas per min, while 1-7 min represent the NTP treatment time.

Postharvest anthracnose disease caused mainly by *C. gloeosporioides* is a major factor which markedly decreases quality and shelf life of mango fruit. To control development of this disease, several alternative methods to fungicides such as heat treatment (Jacobi and Giles, 1997), edible coating (Zhu *et al*., 2008), 1-methylcyclopropene treatment in combination with controlled atmosphere storage (Sivakumar *et al*., 2012) and application of chemicals (Zhang *et al*., 2013; Thinh and Kunasakdakul, 2013) have been investigated. In this study, applied NTP at 5 L/min of Ar flow rate for 7 min effectively reduced the growth of *C. gloeosporioides* in both

in vitro and *in vivo* tests. The obtained results can demonstrate the potential of certain atmospheric NTP application against mold spoilage for heat sensitive fresh produces like Nam Dok Mai mango.