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Article

Influence of Cold Atmospheric Plasma Treatment on Fresh-Cut Mango Shelf-Life Extension

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Abstract. Emergence of cold plasma technology has demonstrated a great potential for decontaminating fresh fruits and vegetables due to its non-thermal characteristics. A packed-bed plasma reactor with maximum high voltage of 8 kVDC was developed to generate gaseous reactive radicals from ambient air for post-process decontaminating of the fresh-cut and ready-to-eat mango. This Cold Atmospheric Plasma (CAP) machine employed gas from the glow discharge and a mixture of this gas with fine mist fog to inactivate microbial load. Average Total Plate Count (TPC) of untreated or controlled fresh-cut mango was observed to be above a maximum TPC requirement of 6 log CFU/g on the 5th day of 4°C refrigerated storage, while those of the CAP treatment reached the maximum TPC requirement on the 10th day. The CAP treatment of fresh-cut mango samples without- and with-fog presented significant microbial reductions of 2.09 and 1.87 log CFU/g, respectively, more than controlled samples on the 10th day of storage. Moreover, a browning-process deceleration of treated mango samples with CAP could be observed from $L^*a^*b^*$ without affecting samples' pH and acidity during 10-day storage. Therefore, the CAP treatment revealed a strong possibility to extend a shelf-life of fresh-cut mango in the refrigerated storage.

Keywords: Cold atmospheric plasma, fresh-cut mango, microbial inactivation, physicochemical attributes, post-process decontamination.

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1. Introduction

Food safety and quality degradation are two main problems of ready-to-eat fruits and vegetables, which become very popular in past few years. After performing minimal processing, fresh-cut fruits and vegetables surface can be contaminated with various pathogens that can cause food-borne illness. For example, *Escherichia coli* and *L. monocytogenes*, commonly found in most fruits and vegetables, can result in symptoms of Shiga toxin-producing *E. coli* (STEC) and Listeriosis infection, respectively. Furthermore, fresh-cut product deterioration; such as, discoloration (or surface browning) and moisture loss (or loss of firmness), can make product less appealing to customers.

Several conventional methods to reduce cross-contamination and to prevent post-process contamination [1] are thermal processing, chemical washing, and edible coating. Fruits and vegetables are heat-sensitive products, thus thermal treatment can lead to texture damage and flavor/nutrition loss. Chemical washing with ethanol or isopropanol for fruits and vegetables is a cost-effective approach for sanitizing and disinfecting harmful microbes; however, chemical residuals could be hazardous for consumers. Other non-thermal methods; such as, UV light, high hydrostatic pressure, pulsed electric field, gamma/beta irradiation are alternative techniques for disinfection of fresh-cut products. To further subside microbial growth of ready-to-eat products, appropriating packaging: modified atmosphere packaging (MAP), can be employed along with storing in cold conditions of refrigeration.

Many research studies have been applied the Cold Atmospheric Plasma (CAP) technology to decontaminate both fresh and fresh-cut fruit and vegetable products [2], [3] so that spoilage microorganisms and pathogenic could be inactivated, for example, the fresh-cut pitaya fruit with CAP treatment at 60 kV for 5 min [4] and fresh-cut cantaloupe under cold plasma at 40 kV for 90 s [5], ready-to-eat rocket leafy salad treated with cold plasma of 6 kV for 10 min [6]. Particularly, bacterial load that is one of the main causes for fresh-vegetable spoilage, could be direct disinfected by the CAP treatment, such as 1) fresh-cut lettuce baby leaves, treated by an atmospheric pressure plasma jet for 15 seconds [7] and 2) tomato-and-lettuce mixed salad, treated with a 35-kV corona discharge plasma for 3 mins [8] and 3) ready-to-eat wine-pickled Bullacta exarate treated with the 40-60 kV DBD for 3 mins [9]. Due to non-thermal properties of cold plasma, no or insignificant effect on nutrition and sensory qualities was observed in the fresh and fresh-cut products, for instance, fresh mangos inside a bath of Sodium bicarbonate solution with cold-plasma discharge of 1.2 kV for 2 mins [10] and red currants inside a diffuse coplanar surface barrier discharge of 300 W for 10 mins [11]. Furthermore, in-package plasma treatment of fresh fruits and vegetables, like spinach, tomato, strawberry [12], and carrot [13] demonstrated a strong potential to preserve food products by decontaminating microbial load inside sealed packages. However, special polymer packaging materials as well as specific design configuration of plasma sources of tens kV must be employed.

Mango is one of the major export products of many developing countries both in Africa and Asia. From 2011-2015, Thailand was among top four countries with high mango trade competition index of each country region, according to UN commodity statistics database [14]. Fig. 1 illustrated a growth of foreign exchange income of Thailand mango trade export along with an increase of mango planting areas over two decades (1996-2015). According to the Department of Trade Negotiation, Thailand was ranked as the seventh world exporter of fresh mangoes in 2021. Several efforts to reduced postharvest losses and food waste of fresh mango were performed to extend its shelf life as well as transformed its into various shelf-stable products, like mango wine/juice and mango leather/power [15] and mango pickles [16]. Thus, prolonging fresh-cut mango shelf life in addition to maintaining its quality and nutrition of fresh-cut mango products after CAP treatment are two major objectives of this research study to lower food waste. Both physicochemical attributes: microbial load and organoleptic qualities: texture, color, and acidity of fresh-cut mango products treated with cold plasma were examined and then compared with controlled product. Section II describes properties of CAP and a developed CAP machine, used in this research. The fresh-cut mango samples and procedures for testing microbe reduction and physicochemical properties during the 10-day storage period are described in Section III. Lastly, the fresh-cut mango products after CAP treatments are compared against those of controlled sets in terms of microbial growth as well as physicochemical effects, which demonstrated a strong potential for extending the fresh-cut mango shelf-life.

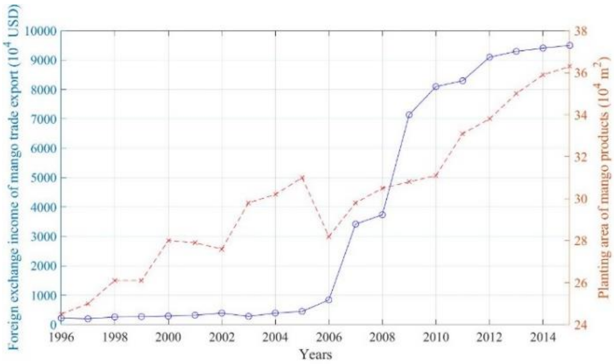


Fig. 1. Foreign exchange income of Thailand mango trade export and Thailand planting areas of mango products increased over the year 1996-2015 [14], according to Ministry of Commerce of Thailand, Statistics Bureau of Thailand.

2. Cold Atmospheric Plasma

Generally, plasma is known as a fourth state of matter, which can be classified either as hot or thermal plasma and as cold or non-thermal plasma. Cold plasma, known as gas discharge, exists in a non-local thermodynamic equilibrium, which can release electrons/particles in a glow-discharge manner. Temperature of heavy particles, including neutrons and protons, of the CAP is near a room ambient temperature (≈300-1,000 K). On the other hand, temperature of electrons inside the CAP is very high (≈10,000 K). Due to its high-energy state, electrons can easily be accelerated in an electromagnetic field, resulting in a flow of electricity through gas/air. During this gas breakdown process, high-energy electrons collide with gas molecules between anode and cathode and create a chain reaction that generates various free radical species of oxygen (or Reactive Oxygen Species – ROS) and of nitrogen (or Reactive Nitrogen Species – RNS). Reactive Species (RS), produced by CAP in gas/air, are composed of short-lived RS and of long-lived RS. The short-lived species, like $HO^{\cdot}$, $HOO^{\cdot}$, $O_2^{\cdot-}$, H^+ [17], from a direct CAP treatment are highly oxidizing and unstable agents that can effectively inactivate pathogen and able to assist in generating the long-lived RS. Furthermore, the long-lived RS, such as O_3, H_2O_2, NO_2^-, NO_3^- are more stable, which can also act as microbial disinfection agents. In additions, UltraVoilet (UV) and visible lights are released as a byproduct of an ionization of gas moleclues.

2.1. Design of Glow Discharge Plasma Reactor

Cold atmospheric plasma was generated inside a packed-bed corona reactor, which is a combination of Dielectric Barrier Discharge (DBD) and the sliding surface discharge. Advantages of this packed-bed corona reactor were the DBD could be operated in a glow discharge mode or Atmospheric Pressure Glow (APG) mode [18] such that homogenous discharge using lower voltage can be achieved without sparks. In this research, the packed-bed corona reactor, characterized by the glow discharge from electrodes, was constructed from 25 copper anodes and 30-plate stainless-steel cathodes, as shown in Fig. 2. As a result, this configuration of 25 electrodes, inserted in the honeycomb-shape Teflon plates as an insulator, can produce total micro-discharge of 750 positions. When the feed gas/air passes through these micro-discharge positions, high-energy free radicals and reactive species can be generated instantaneously and released into a disinfection container. Thus, this setup of the low-pressure cold plasma was more compact than the vacuum cold plasma and radical species can be directly sprayed onto target surfaces to inactivate microorganisms during sterilization processes. Figure 3 illustrates glow discharge or AGP that was generated inside the plasma reactor that could be viewed at the bottom and at the back of the honeycomb-shape reactor.

2.2. Development of Cold Atmospheric Plasma Machine

Efficacy of microbial inactivation with gaseous CAP depends on various parameters, for example, high-voltage power supplied to electrodes of the plasma reactor, working gas to produce cold-plasma reactive species, treatment time, and air-flow rate through the packed-bed corona reactor. Therefore, parameters of the CAP machine must be fine-tuned before performing CAP treatment with the fresh-cut mango samples.

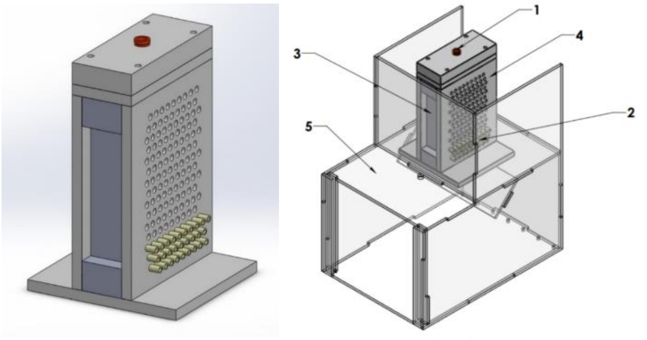


Fig. 2. Twenty-five cold atmospheric plasma electrodes installed inside the packed-bed plasma reactor. (Left) and the CAP treatment apparatus for fresh-cut mango treatment consists of 1) air-flow inlet, 2) copper anode, 3) stainless-steel plate cathode, 4) honeycomb-shape Teflon plate, and 5) disinfection container (Right).

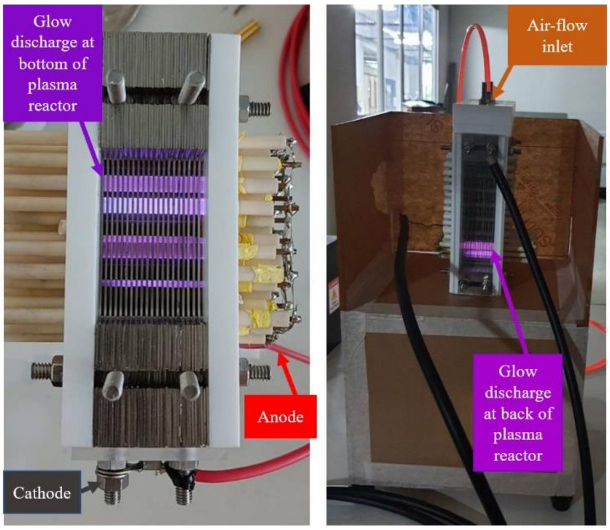


Fig. 3. Glow discharge or AGP was generated at the bottom of the honeycomb-shape plasma reactor (Left) and Glow discharge or AGP appeared at the back of the plasma reactor, installed on the disinfection container (Right).

The CAP machine, developed in this research and illustrated in Fig. 4, was composed of 1) the packed-bed plasma reactor, installed on top of the disinfection container, 2) a high-voltage DC power supply that could generate a maximum voltage of 8 kVDC with a maximum frequency of 8 kHz and a maximum current of 125 Amp, 3) a mist fog machine, which could produce fine water droplets with diameter less than 1 mm from drinking water, 4) a gas-suction system to transfer residual gaseous ozone inside the disinfection container after CAP treatment into a water bucket. Current of the high-voltage power supply through the plasma reactor was kept at 80 mA to avoid overheat of electrodes during continuous CAP treatments. Moreover, an ozone-catalyze machine was also placed next to the disinfection container as an additional safety precaution to accelerate the decomposition of ozone gas that dispersed out of the container during the CAP treatment. To validate the pathogenic inactivation efficacy, a PONPE 311-O3 ozone detector, which could measure ozone concentration between 0 and 20 ppm, was placed inside the disinfection container to measure concentration of ozone gas, which is one of the long-lived stable RS. Figure 5 illustrates a rate of ozone-concentration increase from 10 to 20 ppm inside the disinfection container for two feed air-flow rate of 2 and 5 L/min. For the 2 L/min air-flow rate, the ozone concentration can rise from 0 to 20 ppm within the first 20 seconds after supplying high-voltage to the plasma reactor. Thus, the air-flow rate of 2 L/min was employed in all CAP treatments.

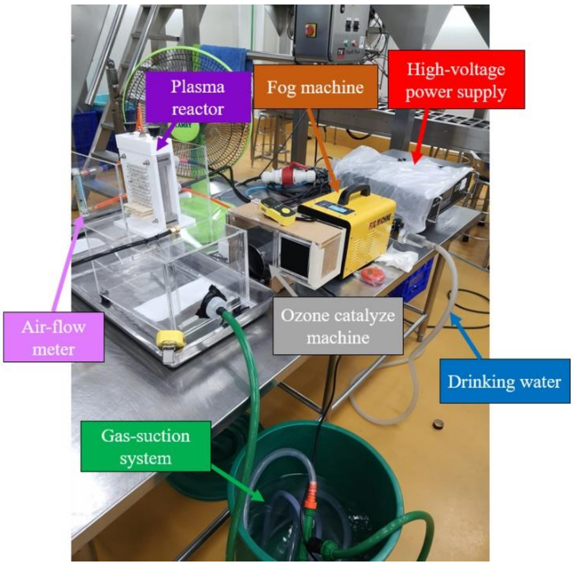


Fig. 4. To perform CAP treatment, the cold atmospheric plasma machine consisted of the CAP treatment apparatus, the high-voltage power supply, the fog machine, the ozone-catalyze machine, as well as the gas suction system to decompose residual ozone in water.

3. Material and Method

This section describes the procedures for assessing microbial reduction as well as physicochemical attributes of fresh-cut mango after the CAP-treatment.

3.1. Fresh-Cut Mango Samples

Mangoes (*Mangiferaindica L., Anacardiaceae)* from Thailand were used in this research study. Mangoes were peeled and sliced into long pieces. They were washed with distilled water to clear dust particles on the peel surface and air-dried at room temperature. Mango slices were placed in a plastic tray with a dimension of 7.5x11x4 cm. During CAP treatments shown in Fig. 6, clean ambient air was fed through the plasma reactor to generate gaseous reactive species that were directly sprayed onto the plastic tray packed with fresh-cut mango slices. In addition, mist fog, generated from drinking water by the fog machine, could be directly sprayed in front of the reactor exit such that free radicals from CAP could be captured inside the water droplet of <1 mm or fine fog. After CAP treatment with- and without-fog and no CAP treatment, each tray was covered with a thick plastic top seal.

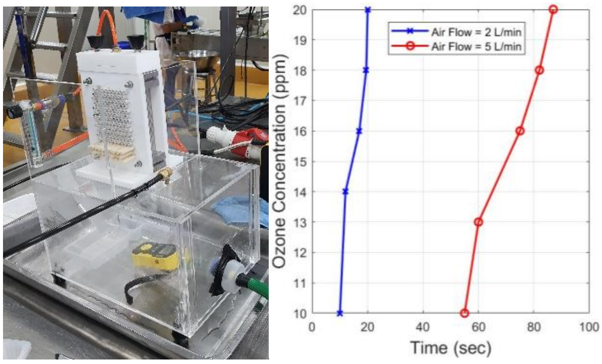


Fig. 5. Ozone concentration inside the disinfection container was measured by the PONPE 311 ozone detector (Left) and graphs of ozone concentration for two air-flow rates of 2 and 5 L/min to validate the effectiveness of gaseous CAP treatment (Right).

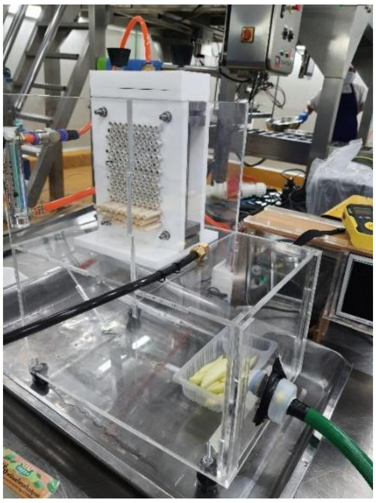


Fig. 6. The CAP treatment of fresh-cut mango samples was performed without fog.

3.2. Procedure for Microbe Reduction Assessment

The fresh-cut mango samples with the CAP treatment as well as controlled (or non-treated) samples, were examined for the total number of microorganisms (FDA, 2001) [19] and for yeasts and molds (AOAC, 2019, method 997.02) [20] during a period of 10 days using the standard methods.

3.3. Procedure for Physicochemical Attribute Measurement

Furthermore, physicochemical properties of the fresh-cut mango, including the titratable acidity and pH, were investigated to validate mango-sensory variation during the storage period of 10 days.

3.3.1. *Titratable Acidity (TA)*: The TA of the fruit pulp was determined by the AOAC (2019), method 942.15 [20].

3.3.2. *pH:* The pH value of each sample was determined by using digital pH meter according to AOAC (2019), method 981.12 [20].

3.3.3. *L*a*b**: The *L**, *a**, *b** color space was measured from the fresh-cut mango sample images in a light-controlled environment. The *L** is the lightness value, where its value ranges between 0 (or black color) and 100 (or white color). The *a** axis ranges between -128 and 127, representing a relative green-red opponent color, respectively. The value of *b** axis varies within -128 and 127, representing a relative blue-yellow opponent color, correspondingly.

4. Results And Discussion

To extend the shelf-life of fresh-cut mango from 5-6 days in the refrigerated storage with food safety in mind, both microbial load reduction and physicochemical properties after the CAP treatment were examined during the 10-day period and discussed in this section.

4.1. Evaluation of Microbe Reduction

The microbial analysis of cold atmospheric plasma-processed fresh-cut mango was evaluated during storage at 4 ˚C. During 10 days of storage, the samples were analyzed every other day, regarding total plate count, total yeast and mold loads. Based on the microbial analysis results during the fresh-cut mango storage, plasma-treated samples seemed to have lower values of microbial load (log CFU/g) for total microorganisms compared to control samples, as shown in Fig. 7. For untreated samples, the total microorganisms were found to increase from 2.33 log CFU/g from day 0 to 7.13 log CFU/g after day 10 of storage at 4 ˚C. For the plasma-treated samples, the count increased from 3.28 log CFU/g to 6.21 log CFU/g and 3.25 log CFU/g to 5.96 log CFU/g when the samples received the 30-sec CAP treatment with and without fine fog, respectively. Due to inevitably initial microbial loading in the fresh-cut mango samples, the total microbial reduction, evaluated based on a difference of final and initial loadings, can be derived as 4.8, 2.93, 2.71 log CFU/g correspondingly for control sets, CAP-treatment sets with fog, and without fog. Approximately 1 log CFU/g reduction was achieved after the plasma process, which was. The results agree with Li *et al.* [4] and Zhou *et al.* [5], who reported a decrease in the microbial count in fresh-cut pitaya fruit and fresh-cut cantaloupe under cold plasma at 60 kV for 5 mins and at 40 kV for 90 seconds, respectively. Moreover, in this study, the effect of the presence of fog on the reduction of Total Plate Count (TPC) was not statistically significant.

For total yeast count, the number of yeasts in cold plasma-treated samples was slightly lower than in the control samples, as shown in Table 1. However, the effect of cold plasma on the mold reduction was excluded in this study. Limnaios *et al.* [11] reported that air-generated CAP by Diffuse Coplanar Surface Barrier Discharge (DCSBD) at 300 W can reduce yeast and mold load of red currants up to 1.28 log CFU/g after 10-min treatment. In the study by Yinxin *et al.* [21], using cold atmospheric pressure plasma can reduce the rot rate of mulberries due to the inhibition of *Botrytis cinereal*, a gray mold causing rot in mulberries. The effect of cold plasma provides the marginal impact on yeast inhibition, demonstrated in this study, this effect might be caused by differences in sources of samples, as well as the parameters relating to plasma-generation conditions such as supplied gas resulting in different generated plasma gases, voltage and configuration of the plasma equipment.

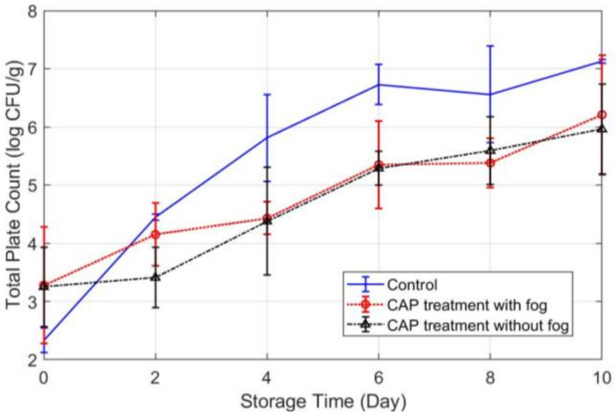


Fig. 7. Total plate count of microbial growth (mean±stdev) in fresh-cut mango samples varied during storage duration between day 0 to day 10 after 30-second CAP treatment with and without water fog and no CAP or control treatment.

Table 1. Analysis of yeast (log cfu/g) on 30-sec cap treatment sets (with and without fog) and control set of fresh-cut mango during 10-day storage at 4°c (mean±stdev).

Storage time (day)	**Control or No CAP treatment**	**CAP treatment with fog**	**CAP treatment without fog**
0	2.72±0.29	2.77±0.21	2.78±0.21
2	4.10±0.14	4.50±0.17	4.03±0.62
4	5.26±1.50	4.63±0.78	4.79±0.95
6	6.43±0.04	6.11±0.00	6.41±0.00
8	6.05±0.09	5.94±0.04	5.85±0.08
10	6.58±0.13	6.51±0.16	6.50±0.06

4.2. Evaluation of Physicochemical Attributes

The cold plasma treatments with and without fog insignificantly affected the change in pH and titratable acidity of fresh-cut mangoes, shown in Tables 2 and 3. These treated mango samples exhibited minor differences in pH and titratable acidity of the untreated fresh-cut mangoes during the storage period of 10 days. Such a finding agreed with Yinxin *et al.* [21] who showed that the pH of fresh mulberries did not alter after the CAP treatment. Limnaios *et al.* [11] also observed that titratable acidity was not substantially different between control and CAP-treatment groups of red currants. Marginal variation in titratable acidity after the cold plasma treatment was also found in blueberries, reported by Sarangapani *et al.* [22]. In contrast, Giannoglou *et al.* [6] reported a pH reduction of rocket leaves after exposing to the cold plasma for more than 10 mins. Interaction between the reactive gases and moisture of food generated acidic compounds, leading to a lower pH value. According to Oehmigen *et al.*, [23], NO- radicals generated during the glow discharge process could form nitric acid, causing the reduction of the pH level.

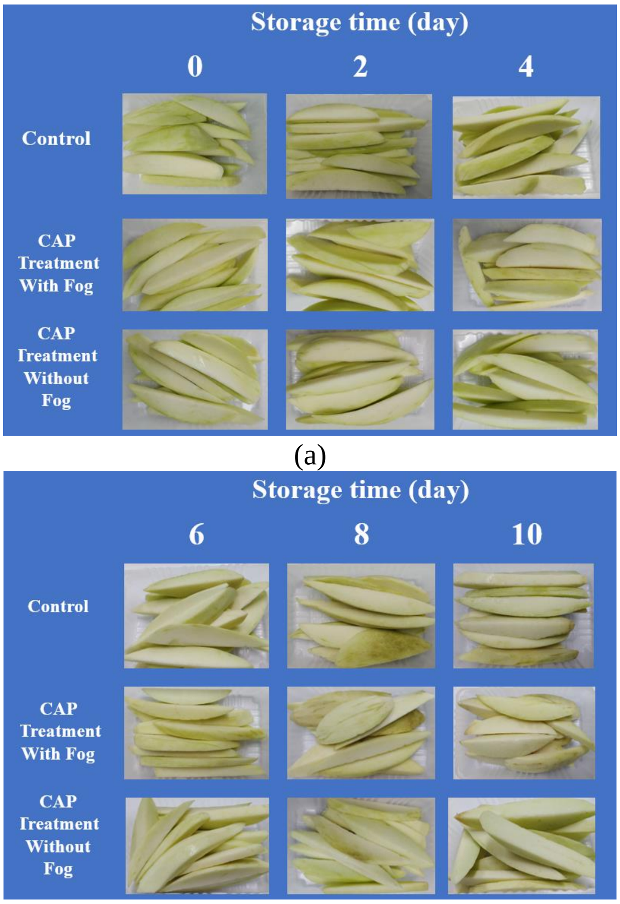
Table 2. Effect of 30-sec CAP treatment on pH of fresh-cut mango samples during 10-day storage at 4°C (mean±stdev).

Storage time (day)	**pH**		
	Control or No CAP treatment	**CAP treatment with fog**	**CAP treatment without fog**
0	3.24±0.08	3.26±0.06	3.26±0.02
2	3.21±0.04	3.21±0.08	3.26±0.14
4	3.33±0.06	3.37±0.11	3.30±0.04
6	3.38±0.02	3.40±0.07	3.31±0.03
8	3.37±0.01	3.34±0.01	3.33±0.04
10	3.33±0.01	3.32±0.08	3.32±0.02

Table 3. Effect of 30-sec CAP treatment on titratable acidity (g of malic acid/100 g of sample) of fresh-cut mango samples during 10-day storage at 4°C (mean±stdev).

Storage time (day)	**Titratable acidity (g of malic acid/ 100 g of sample)**		
	Control or No CAP treatment	**CAP treatment with fog**	**CAP treatment without fog**
0	1.22±0.01	1.25±0.01	1.22±0.01
2	1.24±0.02	1.24±0.01	1.19±0.01
4	1.21±0.01	1.24±0.04	1.25±0.01
6	1.23±0.02	1.24±0.03	1.26±0.02
8	1.25±0.00	1.25±0.01	1.23±0.03
10	1.39±0.06	1.38±0.21	1.44±0.03

In addition to the microbial load reduction using the cold plasma, endogenous enzymes, specifically peroxidases and polyphenol oxidase, that are responsible for creating surface-browning reactions can be subsided by the cold plasma treatment [24]. According to Rico *et al.*, [25], both appearance and color of the fresh-cut products, directly reflected the products' freshness, were two main judgment criteria for product selection by consumers. The appearance of mango slices with the CAP treatment and control sets during the 10-day period are illustrated in Fig. 8 after taking out of the 4 ˚C refrigerated storage. After 8-day storage, mango samples of the controlled set turned brown, while the browning discoloration of the CAP-treatment samples, occurring on the day 10, can be extended. To qualitatively quantify the appearance change, Fig. 9-11 display the variations of *L** (lightness), *a** (redness: green to red), and *b** (yellowness: blue to yellow) during this 10-day period. One of the challenges regarding to the shelf-life extension of fresh-cut fruits was the enzymatic browning reaction. The discoloration of the sample is also confirmed by an increase of redness (*a**) in Fig. 10, coinciding with a decrease in lightness (*L**) in Fig. 9, which represented the presence of browning pigments, reported by Romero *et al.* [26].



(a)

(b)

Fig. 8. The appearance of fresh-cut mangoes after 30-sec CAP treatment with and without fog and no CAP treatment during storage at 4 ˚C from day 0 to 4 (a) and from day 6 to 10 (b).

Research studies by Tappi *et al.* [27], Ramzzina *et al.* [28], Xu *et al.* [29], Buβler *et al.* [30], Yi *et al.* [31], and Abedelmaksoud *et al.* [32] reported the ability of cold plasma to inactivate deteriorative enzymes, such as polyphenol oxidase and peroxidase: key enzymes involved in the browning of fresh fruit and vegetable products. Likewise, treating mango pulp with dielectric barrier discharge plasma (DBDP) for a 10-min duration resulted in a reduction of polyphenol oxidase (10.85%), peroxidase (5.15%), and pectin methyl esterase (5.25%) activities. The mechanism of enzyme inactivation by cold plasma was well-reviewed by Mayookha *et al.* [33]. Two possible mechanisms were 1) the chemical modification and molecular degradation of amino acids by plasma-induced reactive radicals and 2) the disruption of the secondary (2°) structure of enzymes by these reactive radicals. According to Takai *et al.* [34], the plasma-induced reactive oxygen species (ROS) or free radicals (OH, O_2-, HO_2 and NO) could cause chemical modification, for example, oxidation, hydroxylation, sulfonation, amidation and ring opening of amino acids, leading to enzyme inactivation. Adsorption of reactive species or excited molecules, for instance, O_3 or $^1O_2$ from CAP could induce molecular degradation of amino acids, studied by Setsuhara *et al.* [35]. Moreover, changes in α-helix and β pleated sheets of enzymes owning to the CAP reactive species also led to a decrease in enzyme activity, reported by Surowsky *et al.* [36].

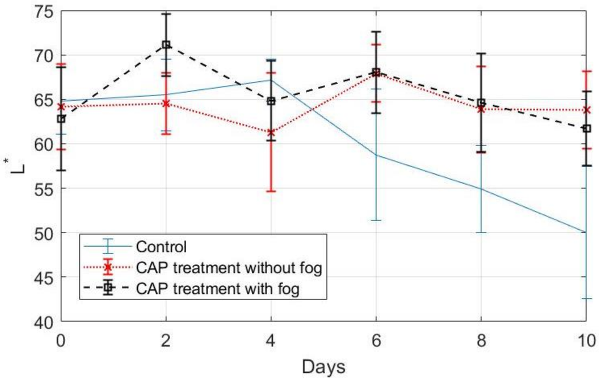


Fig. 9. The L^* color values of fresh-cut mango samples from 30-second CAP treatment without and with fog show the reduction of surface browning comparing against the control set during the 10-day storage period.

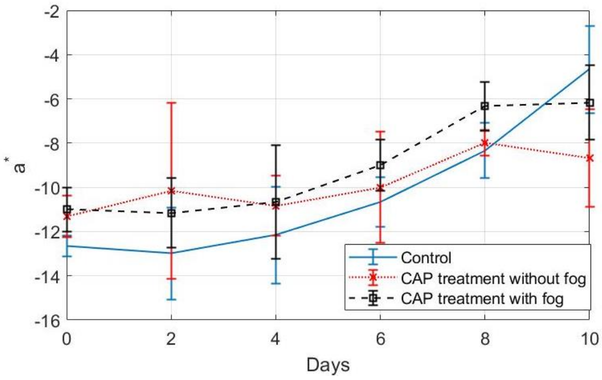


Fig. 10. The a^* color values of fresh-cut mango samples from 30-second CAP treatment without and with fog show the reduction of surface browning comparing against the control set during the 10-day storage period.

5. Conclusion

The Cold Atmospheric Plasma (CAP) machine with the packed-bed plasma reactor with maximum high-voltage of 8 kVDC was developed in this research for treating the fresh-cut mango samples to inactivate microbial load so that the shelf-life of fresh-cut mango samples in the 4°C refrigerated storage can be extended. Clean air was directly fed into the cold-plasma reactor to generate various reactive species with minimum operating cost. During performing the CAP treatment, the safety of operator must be monitored for leakage of ozone gas, one of the long-lived stable RS, from the CAP machine, which could be captured by the ozone catalyze machine and gas-suction system. Moreover, the proper treatment condition using gaseous cold-plasma radicals was experimentally chosen to use the air-flow rate of 2 L/min and the supplied current of 80 mA by the high-voltage power supply.

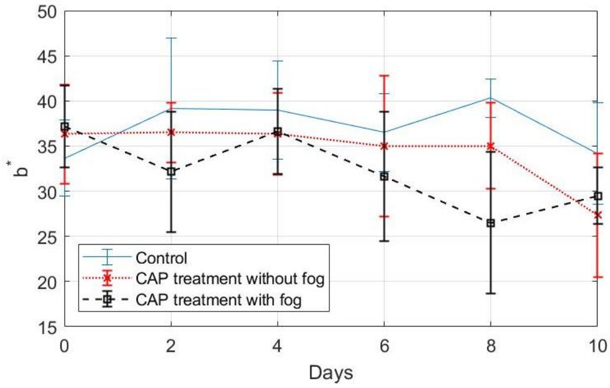


Fig. 11. The b^* color values of fresh-cut mango samples from 30-second CAP treatment without and with fog show the reduction of surface browning comparing against the control set during the 10-day storage period.

Evaluation of fresh-cut mango samples on the microbial load reduction was found that mango samples treated with 30-sec CAP without- and with-fog could delay the growth of microorganisms by 2.09 and 1.87 log CFU/g, respectively, comparing to the control set on the day 10. The maximum requirement for TPC of mango samples should be below 6 log CFU/g, thus cold plasma treatment can provide a strong potential to extend the shelf-life in the refrigerated storage up to 4 days. Specified conditions of CAP treatments with- and without-fog in had no significant impact on yeast and mold inactivation, titratable acidity, and pH level of the mango samples during the 10-day storage period. However, the browning of fresh-cut mango could be decelerated by the CAP treatment, which demonstrated by increase of a^* and decrease of L^* especially for the mango samples, treated with CAP without-fog, at a slower rate than those without any treatment.

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