



Combined antibacterial activity of green tea extract with atmospheric radio-frequency plasma against pathogens on fresh-cut dragon fruit

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ABSTRACT

The objective of this research was to observe the combined effect of green tea extract (at a tea: water; g ml⁻¹, ratio of 2.5–10.0%) and cold plasma (20 W and 40 W) against bacteria pathogens (*Escherichia coli*, *Salmonella typhimurium*, and *Listeria monocytogenes*) that can be found on the fresh-cut dragon fruit. The change in pathogens after plasma treatment of a fresh-cut dragon fruit treated with green tea at 4 ± 1 °C was also investigated. Dragon fruit's nutritional value, mineral, total phenolic content, and sensory within and without plasma and green tea was determined. It was found that atmospheric radio-frequency (RF) plasma at 40 W could extend the protection against all pathogen growth on the surface of fresh-cut dragon fruit treated with at a 5.0% of green tea to at least 15 days. Without the plasma treatment, green tea of 2.5–10.0% could not inhibit all bacterial growth. In addition, higher values of total phenolic content, crude protein, crude fat and crude fibre were observed in the fresh-cut dragon fruit with green tea after the plasma ($p < 0.05$) treatment; however, no change was found ($p > 0.05$) regarding the minerals Fe, Cu, and Mn and the sensory test. The research indicated that green tea extract and atmospheric RF plasma in combination could protect against the growth of pathogens on fresh-cut dragon fruit and extend its shelf-life.

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1. Introduction

Dragon fruit (*Hylocereus*) is a fruit of the cactus species and is one of the ingredients used in many foods such as sherbet, syrup, ice cream (Siddiq and Nasir, 2012). According to its pigment, dragon fruit is red, yellow or white under its skin (Grimaldo-Juarez, Terrazas, Garcia-Velasquez, Cruz-Villagas, & Ponce-Medina, 2007). Betacyanin, for example, is found in red dragon fruit (Jamaludin, Ding, & Hamid, 2011). Fresh-cut dragon fruit or dragon fruit juice contains polyphenol and antioxidant substances (Nur'aliaa, Siti Mazlina & Taip, 2011; Rebecca, Boyce, & Chandran, 2010) which nutritionally benefit human health. Unfortunately, contaminants of bacterial pathogens such as *Salmonella* spp. (Sim, Hong, Yoon, & Yuk, 2013), *Escherichia coli*, and *Listeria monocytogenes* (Corbo, Campaniello, D'amato, Bevilacqua, & Sinigaglia, 2005) on fresh-cut fruit and fruit products have been reported. These harmful

bacteria pathogens can seriously affect humans. Although nutrient loss could occur, preservative treatment for fresh-cut fruit is recommended. In this study, cold atmospheric RF plasma and green tea extract were employed to kill the bacterial pathogens found on fresh-cut dragon fruit.

Green tea is rich in polyphenols, mainly catechins and catechin derivatives including (–)-epigallocatechin-3-gallate (EGCG), epicatechin (EC), (–)-epigallocatechin (EGC), (–)-epicatechin-3-gallate (ECG) and (–)-galocatechin gallate (GCG) (Tsai, Hsu, Ting, Huang, & Yen, 2013). Epigallocatechin gallate (EGCG) is the most common substance in green tea. Studies have reported that EGCG adds to antibacterial activity (Almajano, Carbó, López Jiménez, & Gordon, 2008). Furthermore, EGCG has been reported to be capable of inhibiting the growth of pathogens and spoilage; examples are *Bacillus*, *Campylobacter*, *Clostridium*, *E. coli*, *L. monocytogenes*, *Pseudomonas*, *Staphylococcus aureus*, *Salmonella* and *Vibrio* (Xi, Liu, & Su, 2012). In an attempt to reduce bacterial growth with plasma treatment, green tea extract was employed in this work.

Atmospheric cold plasma can be created by the excitation of gas with electrical discharges at atmospheric pressure at room

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temperature (Tendero, Tixier Tristant, Desmaison, & Leprince, 2006). Radio frequency (RF) or a microwave could be used as the power supplier to produce atmospheric cold plasma or a plasma jet (Herrmann, Henins, Park, & Selwyn, 1999; Park et al., 2003). This type of plasma has benefits for industries or businesses because it contains a low temperature ($<50^{\circ}\text{C}$) that can produce plasma without having to use vacuum equipment. In addition, using low temperature cold atmospheric plasma for food sterilization purposes is recommended (Suhem, Matan, Nisoa, & Matan, 2013a) because it can preserve food nutrition (Suhem, 2013) and process a cooked food as like serves the fresh food for consumer. Recently, plasma has been recognized for its inactivation of many bacteria types such as *E. coli*, *Micrococcus luteus*, *S. typhimurium*, and *S. aureus* (Matan, Matan, & Nisoa, 2014; Yu, Huang, Hsieh, Huff, & Duan, 2007). And very few studies have indicated the combined effect of green tea extract and plasma for inhibiting *S. typhimurium*, *E. coli*, and *L. monocytogenes* on fresh-cut dragon fruit; therefore, the objective for this study was to investigate these claims.

2. Materials and methods

2.1. Fresh-cut dragon fruit preparation

Dragon fruit (*Hylocereus undatus*) was purchased from the Thasala district of Nakhon Si Thammarat, Thailand. The fruit was cut into a width of 3 cm, a length of 3.5 cm and a height of 0.5 cm by using a sterilized knife. It was then kept at 4°C in a refrigerator (Sanyo Co., Ltd, Bangkok, Thailand) until being analyzed.

2.2. Atmospheric RF plasma apparatus

The atmospheric RF plasma jet system used in this study was developed by the Plasma Agricultural Application Laboratory, the Green Innovation in Physics for Agro-industry Research Center of Excellence, Walailak University. Radio frequency (RF) was used for power and adjusted from 20 W to 40 W and 20 kHz–600 kHz. Argon was selected for gas discharge. A diagram of an atmospheric RF plasma jet is shown in Fig. 1.

2.3. Preparation of bacterial cultures

E. coli, *Salmonella typhimurium*, and *L. monocytogenes* were obtained from the Food Microbiology Laboratory of Walailak University. Each strain was grown on nutrient agar (Merck Ltd, Thailand) at 35°C for 24 h. The bacterial population in all of the inoculated media was standardized to 10^6 cells ml^{-1} by dilution with peptone water. The viability of all strains was checked by using quantitative colony counts at 10^6 cfu ml^{-1} .

2.4. Preparation of green tea extract

Green tea leaves (*Camellia sinensis*) were obtained from Chiang Mai, Thailand. Green tea leaf extract was prepared by adding at a tea: water; g ml^{-1} , ratio of 2.5–10% of boiling water for 10 min. The extraction solution was cooled to room temperature and then filtered.

2.5. Effect of atmospheric RF plasma and green tea extract on the surface of the fresh-cut dragon fruit

One ml of each bacteria (*E. coli*, *S. typhimurium*, and *L. monocytogenes*) was first inoculated on the surface of the fresh-cut dragon fruit (3 cm width, 3.5 cm length and 0.5 cm height) ($n = 5$). Specimens were then placed in a sterilization container and were put into a biological safety cabinet (Cellgard, NuAire, USA) to be dried out. During this phase, after 5 h and with the temperature at 30°C , the bacteria infected the dragon fruit's surface. The fruit was next dipped into green tea extract (ratio of 2.5, 5.0, 7.5 and 10.0%) for 60 s. Dip-treated fruit was placed in a container and put into a biological safety cabinet to be dried out at 30°C for 1 h. After drying, the green tea extract treated dragon fruit was subsequently exposed to atmospheric RF plasma at 20 W and 40 W for 60 s. The controls (with only green tea extract (2.5–10.0%) treatment, with only plasma (20 W and 40 W) treatment and without green tea extract and plasma treatment) were also incorporated. For counting the visible bacteria cells, 25 g of the treated dragon fruit or the control specimens were placed into sterile stomacher bags with 225 ml of buffered peptone water (BPW; Nissui Pharmaceutical CO., LTD, Tokyo, Japan). Then, each count of the bacteria was done by using compact dry EC (*E. coli* and *coliforms*), SL (for *Salmonella*) and LS (*L. monocytogenes*) (Nissui Pharmaceutical CO., LTD, Tokyo, Japan) to count the *E. coli*, *S. typhimurium*, and *L. monocytogenes* colonies, respectively. For pre-enrichment of *S. typhimurium*, and *L. monocytogenes*, after homogenized, samples were incubated for 24 h at $35 \pm 2^{\circ}\text{C}$ (Binder, Tuttlingen, Germany). Then, each sample was transferred into compact dry SL and LS for counting, respectively.

2.6. Total phenolic content

Treated fresh-cut dragon fruit (dipped into ratio of 5.0% of green tea extract before being treated with 40 W plasma) and three controls (with only 5.0% green tea extract treatment, with only 40 W plasma treatment and without both green tea extract and plasma treatment) were prepared for its total phenolic content measurement by using a modified version of Folin-Ciocalteu's method (Nurliyana, Syed Zahir, Mustapha Suleiman, Aisyah, & Kamarul Rahim, 2010). Specimens of the dragon fruit ($n = 5$) were added in the Folin-Ciocalteu reagent mixed with the sodium carbonate. The reaction mixture was allowed to react in the dark at 30°C for 30 min. Then the absorbance at 765 nm was measured by a spectrophotometer (Thermo Fisher Scientific, USA). The control blank was done in the same way but deionized water was used to calculate the total phenolic content. The standard curve of gallic acid with concentrations of 0.05, 0.10, 0.15, 0.20, and 0.25 mg ml^{-1} was prepared. Results were expressed in mg 100 g^{-1} .

2.7. Detection of bacteria in naturally infected fruits and fruits inoculated with bacteria pathogen during storage

For the naturally infected fruits, fresh-cut dragon fruit was dipped in green tea extract (ratio of 5.0%) for 60 s and then was treated by using atmospheric RF plasma at a power of 40 W for

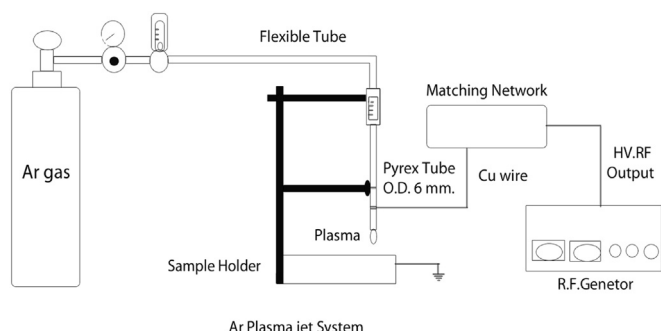


Fig. 1. Schematic view of an atmospheric RF plasma.

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