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Efficacy of ozone application and disinfection treatments on pathogens in fresh-cut vegetables

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Abstract

Consumers and regulators are concerned about the safety of fruits, vegetables, salad, and other ready to eat (RTE) products. This includes preventing contamination from both pathogens and chemical contaminants. Novel industrial applications and improvements in ozone technology were used together with new regulatory actions. Ozone does not leave any residues on the treated produce and it is also accepted by many organic grower organizations. The purpose of this research was to investigate the disinfection efficacy of ozone (O(3)) (2 ppm, 15 and 30 min), water (10 min), and with other four disinfectant: 0.75% normal saline, 3% acetic acid, 0.375% sodium bicarbonate, and 0.1% potassium permanganate with contact time for 10 min for reducing pathogen of fresh-cut kale, cantonese, cauliflower, red chili, and yard long bean. Furthermore, the decontamination efficacy and the effect of these technologies on physicochemical quality of vegetable samples were analyzed. Vegetable quality throughout the storage period (12 days) was determined to employ color and microbiological indexes, such as *Salmonella* spp. and *Escherichia coli*. O(3) was effective disinfection treatments on vegetable wash water, with a maximum microbial reduction of 1.0-2.0 log CFU mL(-1) after 15 min of 2 ppm of ozone treatment. The advantages of this method are the use of much lower sanitizer doses and reduce the frequency of changing the water in the washing step at the packing house.

Keywords: Ozone, Normal saline, Acetic acid, Sodium bicarbonate, Potassium permanganate

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1. Introduction

All types of fresh-cut vegetable have the potential to harbor pathogens, such as *Escherichia coli*, *Listeria monocytogenes*, *Shigella* spp., and *Salmonella* spp., which are ultimately responsible for the majority of foodborne outbreaks (Abadias *et al.,* 2008). It is well known that disinfection represents one of the most critical processing steps influencing the quality, safety, and shelf-life of fresh-cut fruits and vegetables. In the fresh-cut industry, chlorine is generally used as a product sanitizer; however, there is a trend in eliminating this substance due to the environmental and health risks associated with the formation of carcinogenic halogenated disinfection by-products (Gil *et al.,* 2006).

Ozone, a powerful oxidant, is effective against various kinds of microorganisms on fruits and vegetables. It decomposes into simple oxygen with no safety concerns about the consumption of residual by-product (Prabha *et al.,* 2015). Due to its high oxidation capacity and microbial inactivation potential, ozone has prevented various kinds of microbial spoilages usually encountered in fruits and vegetables. The problems of the food industry like mycotoxin and pesticide residues can be reduced by ozone application. If improperly used of ozone concentration, ozone can cause some deleterious effects on products, such as losses in sensory quality (Karaca and Velioglu, 2007). The most notable effect of ozone on sensory quality of fruits was the loss of aroma. Ozone-enriched cold storage of strawberries resulted in reversible losses of fruit aroma (Nadas *et al.,* 2003). If used in proper conditions, ozone may prevent microbial spoilage and some diseases, with a minimum amount of physiological damage to fruits and vegetables. Ozone treatment assures the retention of sensory, nutritional and physicochemical characteristics of food. Treatment conditions should be specifically determined for all kinds of products for effective and safe use of ozone (Prabha *et al.,* 2015). These conditions must be specifically determined by trials of ozone application to all kinds of products.

Koseki and Isobe, 2006 found that the combined treatment of hot water (50°C, 2.5 min) followed by ozonated water (5 ppm, 2.5 min) had the same bactericidal effect as treatment of lettuce with ozonated water (5 ppm, 5 min) or sodium hypochlorite (NaOCl, 200 ppm, 5 min), giving a reduction in bacteria numbers of 1.2 to 1.4 log CFU/g. The ascorbic acid content of the lettuce was not affected by these treatments. The concentration of ozone reflects the extent of browning, increased dramatically in lettuce treated with 10 ppm ozonated water compared with other treatments. Furthermore, using low level of dissolved ozone (1.4-2.0 mg/L) for removal of the four pesticides residue: methyl-parathion, parathion, diazinon and cypermethrin on vegetable surface (Brassica rapa) (Wu *et al.,* 2007). Moreover, the spoilage microorganisms of vegetables such as onions, potatoes, and sugar beets are also

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reduced after storage in an atmosphere containing low ozone concentrations (Gil and Selma, 2006).

The effect of ozonated water treatment on the quality and safety of fresh produce compared with that of the water wash treatment and household sanitizers has not been reported. The present study evaluated the efficacy of ozone (O(3)) 2 ppm, 0.75% normal saline, 3% acetic acid, 0.375% sodium bicarbonate, and 0.1% potassium permanganate for the inactivation of microorganisms (*Salmonella* spp. and *E.coli*) on the surface of fresh produce. The findings will help extend the shelf life and visual quality of fresh−cut kale, cantonese, cauliflower, red chili, and yard long bean in order to help support the quality and safety of fresh produce in Thailand.

2. Materials and Methods

2.1 Sample preparation

Fresh−cut kale, cantoneese, cauliflower, red chili, and Long yard bean were purchased from the fresh market at Mae Hia district, Chiangmai, Thailand. Non−edible parts of samples were trimmed out and washed with tap water to remove soil and other contaminated particles. *Escherichia coli* and *Salmonella* spp. were cultured in Tryptic Soy Broth (TSB) at 37°C for 18 hr (log phase state). All vegetable samples were artificially contaminated by inoculating with certain concentration (10^3 cfu/mL) of 10 μL of *E.coli* and *Salmonella* spp. on each vegetable sample (100 g) for 10 points and left for one hour before analyzing it in the next step. (Paul and Kevin, 2009). The concentrations of 0.75% normal saline, 3% acetic acid, 0.375% sodium bicarbonate, and 0.1% potassium permanganate were prepared with following method of the Thai department of health, 2013. The effects of these methods for reducing *E.coli* and *Salmonella* spp. on fresh−cut kale, cantoneese, cauliflower, red chili, and Long yard bean were carries out.

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2.2 Comparative study of efficiencies of disinfection treatments on vegetable samples

Full Factorial in Completely randomized design was the experimental design used in this study (6 x 3 x 2 = 36 samples). Samples of each vegetable (100 g) were treated with 2 ppm gaseous ozone for 15 and 30 min and washed with tap water for 1 min (Wu *et al.,* 2007). One hundred grams of each vegetable contaminated with pathogens were dipped with water and four different disinfectants for 10 min: 0.75% normal saline, 3% acetic acid, 0.375% sodium bicarbonate, and 0.1% potassium permanganate and washed with tap water for 1 min, each vegetable (100 gram) was dipped with distilled water for 10 min as the control. After dipping, the samples were drained for 5 min and all experiments were analyzed in triplicate. The viable numbers of *E.coli* and *Salmonella* spp. were determined in triplicate following the method of BAM (2019). The total of 36 samples of each vegetable sample were randomly collected and checked by visual examination. Then *E.coli* isolations were done by using Eosin Methylene Blue (EMB) agar whereas *Salmonella* spp. was isolated using xylose–lysine deoxycholate agar (XLD) and modified semi–solid rappaport vassiliadis agar (MSRV). The positive results were subsequently confirmed with biochemical tests.

2.3 Study of Physico–chemical and quality changes of vegetable samples treated with ozonated water

Quality of fresh–cut kale, cantoneese, cauliflower, red chili and Long yard bean samples treated with ozone were investigated at day 0, 3, 6, 9, 12, and 15 of chilling storage with the controlled temperature at 4 ± 2°C and controlled relative humidity at 85 ± 5%. All vegetable were collected for measurement of weight, color, and physical characteristic by using a digital weighing scale, Hunter scale lab, and digital camera, respectively. The most effective method in reducing of pathogens by ozone treatment was chosen regarding the calculating of the percentage of positive samples and concentration of pathogens align with Thai agricultural commodity and food standard TACFS 9007–2005: Safety requirements for agricultural commodity and food (Thai ACFS, 2005)

3. Results and discussion

3.1 Efficiencies of disinfection treatments on vegetable samples

Colony forming unit (CFU) of *E.coli* and *Salmonella* spp. obtained from ozone exposed samples were significantly reduced compared with other disinfectants. No *E.coli* and *Salmonella* spp. colonies were isolated from non–inoculated vegetable samples. The samples artificially contaminated with *E. coli* and *Salmonella* spp. treated with 2 ppm ozone for an exposure time of 15 and 30 min showed a 1–log reduction in colony count compared with the untreated control (water wash treatment) (as shown in Table 1)

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Table 1 The viable numbers of pathogens contaminated on vegetable samples after various disinfection treatments.

Treatment	Vegetable	*E. coli* *(CFU/g)*	***Salmonella*** **spp.** *(CFU/g)*
Ozone 15 min	Kale	1.33E+05	3.88E+04
	Cantonese	3.00E+05	2.90E+05
	Cauliflower	4.63E+04	2.75E+04
	Red chili	1.22E+04	0.00E+00
	Long yard bean	2.45E+04	5.50E+04
Ozone 30 min	Kale	1.52E+05	4.43E+04
	Cantonese	3.24E+05	7.50E+04
	Cauliflower	7.60E+04	1.60E+05
	Red chili	4.55E+03	7.25E+03
	Long yard bean	3.29E+04	3.68E+03
Water 10 min	Kale	3.03E+05	7.05E+04
	Cantonese	1.39E+05	1.62E+05
	Cauliflower	2.95E+04	3.30E+03
	Red chili	9.53E+04	1.45E+05
	Long yard bean	4.20E+04	2.33E+05
0.75% Normal saline 10 min	Kale	9.80E+04	1.92E+05
	Cantonese	2.05E+05	1.82E+05
	Cauliflower	4.60E+04	3.64E+04
	Red chili	2.91E+04	2.38E+03
	Long yard bean	6.39E+04	9.61E+04
3% Acetic acid 10 min	Kale	1.27E+05	3.13E+04
	Cantonese	1.30E+05	2.42E+05
	Cauliflower	1.30E+04	2.30E+04
	Red chili	2.53E+04	6.30E+03
	Long yard bean	1.10E+05	1.03E+05
0.375% Sodium Bicarbonate	Kale	2.55E+04	1.71E+05
10 min	Cantonese	1.60E+05	2.77E+05
	Cauliflower	4.05E+04	7.34E+04
	Red chili	5.70E+03	8.90E+03
	Long yard bean	1.58E+05	3.90E+04
0.1% Potassium permanganate 10 min	Kale	5.67E+04	3.55E+03
	Cantonese	1.70E+05	6.03E+04
	Cauliflower	5.00E+02	1.25E+02
	Red chili	1.04E+05	9.24E+04
	Long yard bean	4.04E+04	1.08E+04

Remark: Results are reported as the mean ± standard deviation of the mean

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E.coli and *Salmonell spp*. were found in all the samples after washing with ozone (O(3)), normal saline, acetic acid, sodium bicarbonate, and potassium permanganate. In study of Horvitz and Cantalejo, 2010, fresh-cut red peppers were washed with chlorinated water (200 ppm) or treated with 0.7 ppm ozone for 1, 3 and 5 min, It found that O(3) was effective disinfection treatments on vegetable wash water, with a maximum *E.coli* and *Salmonella* spp. reduction of 1.0 and 2.0 log CFU mL(-1), respectively of ozone treatment. Microbial growth on fresh produce treated with sanitizer is more rapid growth than untreated samples. This results was similar to the study of Koseki and Isobe, 2006, bacterial populations on the lettuce treated with sanitizers were initially reduced but then showed rapid growth compared with that of the water wash treatment, which did not reduce bacterial counts initially because of an initial decrease in the bacterial population, which reduces the number of competing bacteria and allows the remaining bacteria to thrive.

3.2 Physico-chemical and quality changes of vegetable samples treated with ozonated water

In table 2, the results indicated that microbial contamination is one of the main causes of vegetables decay during storage (Horvitz and Cantalejo, 2010). No visual ozone damage was observed when vegetables were treated with 2 ppm ozone for 15 min on the first day of storage. Treatment with ozone also suppressed increases in the a* value, thus retarding the progress of browning throughout the 3 day of storage. Despite the weight loss, overall quality was not affected. The results obtained in visual overall quality (Fig 1, Day 0 and Day 3) demonstrated that all vegetable samples maintained very good visual until day 3 of storage at 4°C.

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Table 2 Effects of disinfection treatments on Physico-chemical properties of vegetable samples on day 0 and day 3

Treatment	Vegetable	Quality	Day O			Day 3		
	Kale	Weight *(g)*	227.30			226.57		
		Color	*L	*a	*b	*L	*a	*b
			58.14	-10.43	14.83	55.97	-9.90	14.52
	Cantonese	Weight *(g)*	192.17			191.85		
		Color	*L	*a	*b	*L	*a	*b
			50.51	-12.32	17.41	51.05	-11.73	17.43
	Cauliflower	Weight *(g)*	142.88			142.65		
Control		Color	*L	*a	*b	*L	*a	*b
			73.12	-2.53	32.77	71.03	-1.63	29.16
	Red chili	Weight *(g)*	195.90			195.61		
		Color	*L	*a	*b	*L	*a	*b
			34.94	35.71	17.14	36.99	23.62	9.05
	Long yard bean	Weight *(g)*	213.57			215.57		
		Color	*L	*a	*b	*L	*a	*b
			52.16	-10.45	17.05	49.00	-9.86	16.77
	Kale	Weight *(g)*	194.67			191.67		
		Color	*L	*a	*b	*L	*a	*b
			53.33	-12.94	16.44	53.52	-10.78	12.24
	Cantonese	Weight *(g)*	240.47			233.63		
		Color	*L	*a	*b	*L	*a	*b
			50.67	-12.38	17.86	48.22	-13.85	20.42
	Cauliflower	Weight *(g)*	136.15					
Ozone		Color	*L	*a	*b	*L	*a	*b
			74.35	-2.95	27.76	73.11	-1.91	24.11
	Red chili	Weight *(g)*	185.34			186.57		
		Color	*L	*a	*b	*L	*a	*b
			34.40	33.72	14.99	37.56	27.88	14.35
	Long yard bean	Weight *(g)*	158.34			160.02		
		Color	*L	*a	*b	*L	*a	*b
			51.98	-9.70	16.83	43.99	-8.12	14.38

The visual appearance and freshness of leafy produce have been the main judging criteria for quality distinction at purchase or consumption. In general, although all samples presented physiochemical changes after 12-d storage period at 4°C, the samples treated with 2 ppm gaseous ozone for 15 min, those characteristics better than control samples. The significant visual blemishes and discoloration of vegetables were observed at 6- and 9-d storage for control and ozone treatment, respectively.

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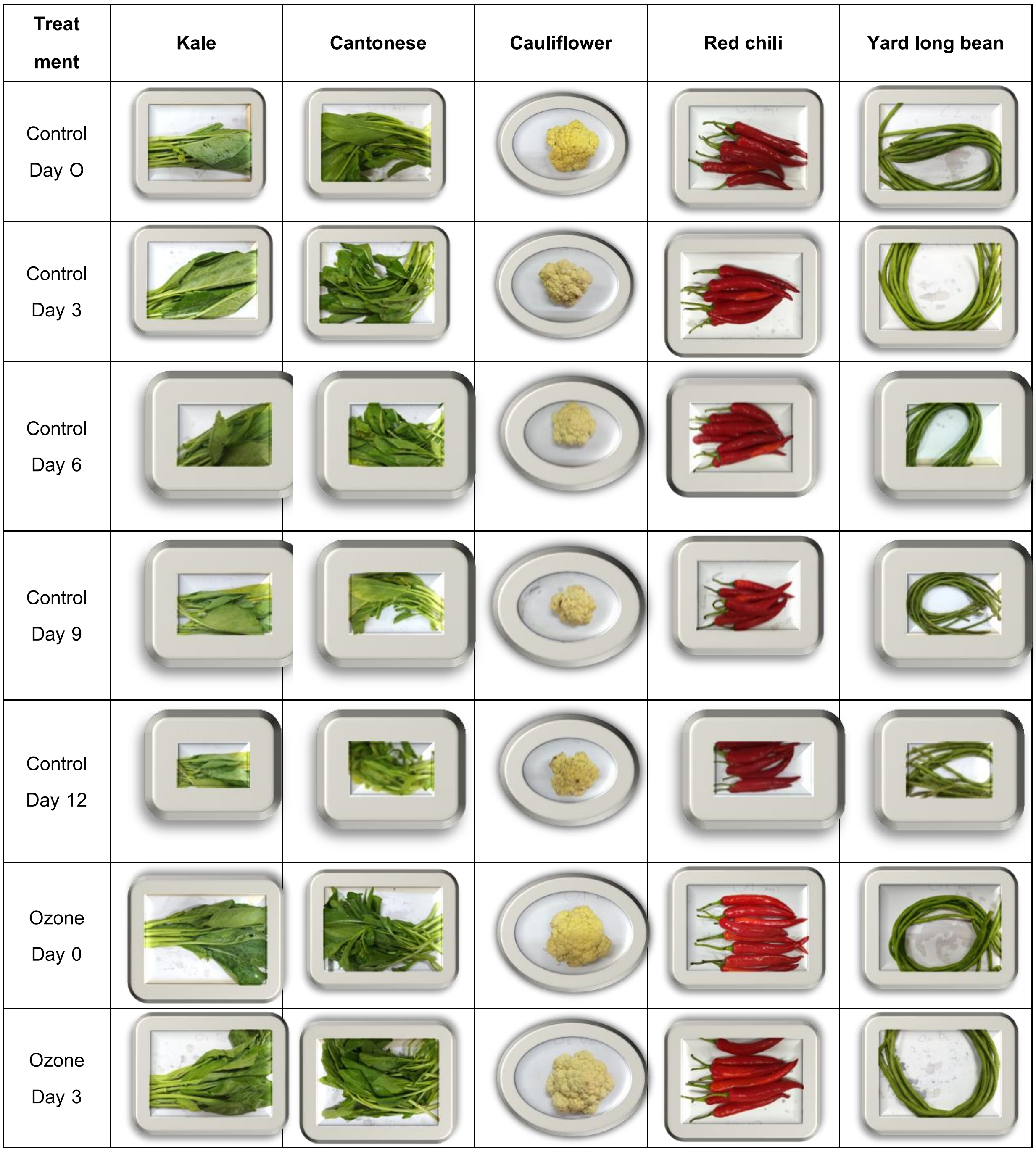

Fig 1 Impact of ozone exposure on the visual quality of fresh-cut vegetables when treated with 2 ppm ozone concentration for 15 min at storage day 0, 3, 6, 9 and 12, with control at storage day 0, 3, 6, 9, and 12

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Treatment	Kale	Cantonese	Cauliflower	Red chili	Yard long bean
Ozone Day 6					
Ozone Day 9					
Ozone Day 12					

Fig 1 Impact of ozone exposure on the visual quality of fresh-cut vegetables when treated with 2 ppm ozone concentration for 15 min at storage day 0, 3, 6, 9 and 12, with control at storage day 0, 3, 6, 9, and 12 continue.

4. Discussion

All sanitizers effectively achieved the same results in terms of initial microbial load. From the results, the potassium permanganate solution at 0.1% concentration for minimum 10 min contact time was proved to be an efficient and easy method to significantly reduce the bacterial load. This can be used as an alternative to or in combination with plain water washing (Subramanya *et al.,* 2018). The use of ozonated water with overflow in the case of vegetable dipping treatment will be required for stable ozone concentration and for stable bactericidal effect. In study of Koseki and Isabe, 2006, the ozone concentration in the ozonated water without overflow decreased by dipping the cut lettuce, the ozone concentration in the ozonated water was stable with overflow.

Consumption of raw fruits and vegetables has been implicated in several food borne illnesses (Estrada−Garcia *et al.,* 2004). Mritunjay and Kumar reported contamination of vegetable salads with pathogens like *Salmonella* and Enterohaemorrhagic *Escherichia coli*. Washing the leafy vegetables with water, supposed to flush out the microorganisms is considered a satisfactory household decontamination method (Subramanya *et al.,* 2018). Many researchers worked on the efficacy of various sanitizers and decontaminants like NaCl solutions, vinegar, combined salt and vinegar, laundry detergent, household bleach products, iodine, trisodium phosphate etc. in various concentrations and with various contact time,

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but none of them could completely eliminate fecal coliform populations from vegetables and fruits (WHO, 1998).

5. Conclusions

The microbiological results obtained with various types of disinfection treatments have shown that the use of ozone is effective in reducing pathogens of the vegetables compared to the untreated control. Exposure to 2 ppm gaseous ozone treatment for 15 min, significantly reduced *E.coli* and *Salmonella* spp. populations on fresh−cut vegetables. In particular, the positive effects of ozonation were appreciable after the sixth day of storage. Moreover, ozone treatment did not affect the color of the product. The role of education, training and awareness among the producers, handlers and consumers is important in order to improve product safety. Washing vegetables before consumption is an important approach for health risk reduction (Subramanya *et al.,* 2018).

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