

Research Article

Effect of Ozone Exposure on Microbial Flora and Quality Attributes of Papaya (*Carica Papaya L*) Fruit

Ong Mei Kying^{1,2*}, Asgar Ali³

¹Department of Agricultural and Food Science, Faculty of Science, UniversitiTunku Abdul Rahman, JalanUniversiti, Bandar Barat, 31900 Kampar, Perak D. R., Malaysia.

²Centre for Biodiversity Research, UniversitiTunku Abdul Rahman, JalanUniversiti, Bandar Barat, 31900 Kampar, Perak D. R., Malaysia.

³Centre of Excellence for Postharvest Biotechnology (CEPB), School of Biosciences, The University of Nottingham Malaysia Campus, JalanBroga, 43500 Semenyih, Selangor D. E., Malaysia.

***Corresponding author:** Ong Mei Kying, Department of Agricultural and Food Science, Faculty of Science, Universiti-Tunku Abdul Rahman, Jalan Universiti, Bandar Barat, 31900 Kampar, Perak DR, Malaysia, Tel.: +605-4688888 ext. 4701; Fax: +605-4661313; E-mail:ongmk@utar.edu.my.

Citation: Kying OM and Ali A (2016). Effect of Ozone Exposure on Microbial Flora and Quality Attributes of Papaya (*Carica Papaya L*) Fruit. J Agr Agri Aspect 2016: JAAA-104.

Received Date: 23 November, 2016; **Accepted Date:** 10 December, 2016; **Published Date:** 17 December, 2016

Abstract

Ozone or triatomic oxygen, is a powerful disinfectant and oxidizing agent. Despite the fact that the application of ozone for the disinfection of fresh fruits and vegetables has been studied widely, comparatively little information is presented on the potential of ozone to reduce microbial populations in papaya fruit. In this study, ozone was applied in gaseous form to papaya fruit at five concentrations (0.05, 0.5, 2.0, 3.5 and 5.8 ppm) for five different periods (0.5, 1, 3, 5 and 24 h) and the reduction in the total bacterial count (coliforms, yeasts and moulds) was assessed. The effect of ozone on weight loss, firmness, fruit peel colour, soluble solids concentration and titratable acidity were also determined. Total mesophilic bacteria on papaya fruit were reduced by 83.3 to 99.7% after exposure to 0.05 to 5.8 ppm ozone for 24 h. Ozone treatments did not affect peel colour and titratable acidity, but significantly reduced weight loss, retained firmness and increased soluble solids concentration during ambient storage. In summary, ozone treatments can be useful for maintaining quality attributes and reducing microbial flora in papaya fruit.

Keywords: Weight Loss; Ambient Storage; Fresh Fruits; Disinfecting Agent; Food Safety

Introduction

In fresh produce industry, the number of illnesses associated with fresh produce is a safety challenges. Fresh produces is often consumed raw or with only minimal processing. Microorganisms, especially bacteria, occur normally on fresh fruit and vegetable tissues. They include mesophilic bacteria, lactic acid bacteria, coliforms, yeasts and moulds (Nguyen-The and Carlin, 1994). Estimated numbers of microbial counts reported on fresh fruits and vegetables are usually within the range 10¹-10⁹ cfug⁻¹, depending upon the fruit or vegetable. The native microbial flora of fruits is mostly composed of moulds and yeasts. Fungi, such as *Botrytis cinerea* and *Aspergillus niger*, and yeasts, such as species of *Candida*, *Cryptococcus*, *Fabospora*, *Saccharomyces* and *Zygosaccharomyces*, are present in most fresh fruits (Chen, 2002).

Microbial spoilage appears to be one of the major reasons for quality loss in fresh fruits and vegetables by development of off-flavours, fermented aromas and tissue decay. The basic principle of shelf-life prediction involves the quantification of populations of microbes present on the food product (Zhuang et al., 2003). In the search for useful disinfectant treatments for fresh vegetables and fruits, decontamination methods include physical (Ukuku et al., 2006; Ozkan et al., 2011; Tzortzakis et al., 2008), chemical (Wang et al., 2007; Maqbool et al., 2011) and biological processes (Rouse et al., 2008; Spadaro et al., 2008). The most versatile treatment is processing with ionizing radiation.

It is a common sanitizing practice of applying cheap and effective synthetic fungicides on papaya, used in combination of pre-pack sanitation treatments employing chlorine- (or bromine-) based disinfectants (Eckert and Ogawa, 1988). Although chemical sanitizer has been used for years, there is much controversial because the residues of it may be harmful to consumers (Hewa-

juligeet al., 2009). Therefore, it is necessary to find a novel way to improve food safety by reducing or eliminating food-related microorganisms during the shelf life of food products without leaving any toxic residues. Sanitation of food with ionizing radiation, such as ozone, is safe, efficient, environmentally clean and energy-efficient. Recently, there has been a high level of interest in postharvest applications of ozone for decay control and as a potential sanitizer against human pathogens because ozone has been affirmed the status of Generally Recognized As Safe (GRAS) as a food processing aid and as compliant with EPA Disinfection by Products Rule (US FDA, 1997).

The application of ozone at the postharvest stage has been studied by many researchers, especially for the prevention of fungal decay (Palou et al., 2002; Perez et al., 1999; Onget et al., 2012), inactivation of bacteria (Achen and Yousef, 2001; Kim and Yousef, 2000; Sharma et al., 2002; Xu, 1999), destruction of pesticides and chemical residues (Onget et al., 1996; Hwang et al., 2001) and the control of storage pests (Kellset et al., 2001; Mendez et al., 2002).

The study of the effect of ozone on papaya fruit is of great importance to determine the potential of ozone as a disinfecting agent and, most importantly, to enhance food safety by not affecting the sensory or nutrient quality. The aim of this work was to evaluate the efficacy of ozone in gaseous form on microbial flora disinfection and quality of papaya fruits.

Materials and methods

Plant material

Mature green papaya cv 'Sekaki' of colour index 2 (green with trace of yellow) were obtained from a local fruit wholesaler at PasarBorong Selangor, Malaysia on the day of their harvesting. Fruit of uniform size (800-1200g), shape, maturity and free from any indication of mechanical injury, insect or pathogenic infection were selected for the experiment. They were washed with distilled water and air-dried at ambient temperature (25-28°C) before exposure to ozone.

Ozone fumigation

The fumigation system, comprised of chambers constructed from 5 mm thick polycarbonate (112.0 cm length x 47.0 cm width x 43.0 cm height) equipped with four 12V fans positioned directly below the sample platform to ensure a well-mixed atmosphere, was set up in the postharvest laboratory, School of Biosciences, University of Nottingham Malaysia Campus. Ozone was introduced to each chamber by an ozone generator (Model MedKlinn Professional Series, MedKlinn International Sdn. Bhd., Malaysia) and the ozone concentration was controlled manually using a sensor (Eco-Sensor, Model OEM-2, MedKlinn International Sdn. Bhd., Malaysia). The ozone concentration in each chamber was

recorded via an ozone analyzer (Model IN-2000 L2-LC, IN USA Incorporated, USA). The chambers were maintained at $25 \pm 3^\circ\text{C}$ and $70 \pm 5\%$ relative humidity (RH), monitored by temperature/humidity sensors (Model U14-001, HOBO LCD Data Logger, Onset Computer Corporation, USA).

Microbial evaluation

Fruit were exposed to ozone enriched atmospheres of 0.05, 0.5, 2.0, 3.5 or 5.8 ppm for durations of 0, 0.5, 1, 3, 5 and 24 h. The experiments were conducted in a completely randomized design and a sample of 48 fruits in each treatment was used. Four fruits from each treatment were used for the microbiological analysis.

Standard plate counts

Microbiological analysis of papaya samples was carried out aseptically according to APHA (1992) procedures by mixing 25 g of papaya fruit skin with 225 ml sterile Ringer solution in a sterile stomacher bag. The samples were massaged by hand for 1 min. The pour plate method was used for total mesophilic bacteria count (Plate count agar, Merck) at 35°C for 24 h. Microbial counts were expressed as log cfug-1.

Yeast and mould counts

The surface spread method was used for yeast and mould enumeration (Yeast Extract Glucose Chloramphenicol Agar, YGC, Merck) at 25°C for 3 to 5 days. Microbial counts were expressed as log cfug-1.

Total coliform counts

The surface spread method was used for coliform bacteria (Mcconkey agar, Merck) at 37°C for 48 hours. Microbial counts were expressed as log cfug-1.

Quality evaluation

All treatments with 24 h exposure were kept for up to 14 days of ambient storage ($25 \pm 3^\circ\text{C}$, $70 \pm 5\%$ RH) during which the quality attributes of the fruit were evaluated. All data were recorded at 2-day intervals. In each treatment, eight fruits were randomly selected for physicochemical analysis.

Physical quality parameters

Fruit in each replication were marked and weighed (using a digital balance, EK-600H, Japan) before storage for weight loss determination. The fruits were then weighed at the 2-day intervals over the storage period. The results were expressed as percentage of initial weight loss.

Fruit firmness was analysed using an Instron Universal Testing Machine (Model 5540, USA). Fruit in each replication were compressed using the 50 mm diameter probe, at a speed of 50 mm

min-1. The compression force measured at the maximum peak of the recorded force was expressed in Newtons (N).

Fruit peel colour was determined using a Minolta CR-300 Chroma Meter (Minolta Corp., Japan). The colour determination made on papaya peel was expressed in chromaticity values of L*, C* and h°. The values of lightness (L*) form the vertical axis with values ranging from 0 = black to 100 = white. Values for a* (red-green axis) and b*(yellow-blue axis) represent coordinates in the colour chart indirectly reflecting chroma (C*) and hue angle (h°). The C* value which refers to the vividness of colour was computed from a* and b*, i.e. $C^* = (a^{*2} + b^{*2})^{1/2}$, which represents the hypotenuse of a right angled triangle with values ranging from 0 = least intense, to 60 = most intense. The h° value was referred to as colour, and was the angle of tangent-1 b*/a*, where 0° = red purple, 90° = yellow, 180° = bluish-green and 270° = blue.

Chemical quality parameters

During storage, the soluble solids concentration (SSC) and titratable acidity of the fruit pulp were determined. Fruit samples from each replication (10 g) were homogenized using a kitchen blender with 40 ml of distilled water, and filtered through muslin cloth. The fruit pulp was sampled from the equatorial region of the whole piece of fruit. A drop of the filtrate was then used to determine the SSC (°Brix) using a Palette Digital Refractometer (Model: PR-32α) from Atago Co, Ltd (Japan) calibrated with distilled water prior to taking readings. The readings were multiplied by the dilution factor to obtain the original SSC (%) of the papaya pulp.

The titratable acidity (TA) was analysed using the titration method described by Ranganna (1999). Ten grams of pulp tissue was homogenized with 40 ml of distilled water using a kitchen blender for two minutes. The mixture was then filtered through muslin cloth. Five ml of filtrate with one to two drops of 0.1% phenolphthalein as indicator was titrated against 0.1N sodium hydroxide (NaOH) to endpoint pink (10 seconds). Titratable acidity was expressed as percentage citric acid equivalents.

Statistical analysis

Treatments were arranged in a completely randomized design. There were four replications for each ozone treatment. A replicate of 12 fruits were placed in a single layer of the chamber. Each chamber was prepared as a replicate. Four fruits were withdrawn at different times to give the 'duration' treatment. Experimental data were analyzed using analysis of variance (ANOVA) via SAS 9.1 software and means were separated using Duncan's Multiple Range Test (DMRT) at (p<0.05). The entire experiment was repeated twice. The results presented in this study were the means of these experiments.

Results and discussion

Standard plate count

Treatment of fruit with ozone at 0.5, 2.0, 3.5 and 5.8 ppm for 24 h reduced the total mesophilic microorganism counts with initial values of 4.48 to 2.18 log cfug-1 (Table 1) (p<0.05 for both the concentration of ozone and the exposure time). These bacteria are commonly found on crop surfaces. Many microbes are capable of colonizing the produce by producing pectin-degrading enzymes which enhance tissue softening and breakdown (Watada et al., 1996). Microbial load in fresh-cut produce is greater than that in intact produce because tissue damage caused by cutting breaks cells and promotes the release of nutrients used by microflora (Toivonen and DeEll, 2002). Ozone is an effective antimicrobial gas due to its oxidizing capacity to kill spoilage and human pathogenic bacteria, yeasts and moulds (Guzel-Seydim et al., 2004). Ozone destroys microorganisms by the progressive oxidation of vital cell components (Beuchat, 1992), preventing the microbial growth and extending the shelf-life of fruit.

	Exposure time (hour)	Ozone concentration (ppm)				
		0.05	0.5	2	3.5	5.8
Total coliforms	0	4.13 ^{Aa}	4.13 ^{Aa}	4.13 ^{Aa}	4.13 ^{Aa}	4.13 ^{Aa}
	0.5	4.01 ^{Bb}	3.48 ^{Cb}	3.33 ^{Db}	3.02 ^{Eb}	2.98 ^{Fb}
	1	3.56 ^{Bc}	3.31 ^{Cc}	2.54 ^{Fc}	2.93 ^{Dc}	2.88 ^{Ec}
	3	2.98 ^{Bd}	2.81 ^{Dd}	2.4 ^{Fd}	2.93 ^{Cc}	2.54 ^{Ed}
	5	2.98 ^{Bd}	2.65 ^{De}	2.18 ^{Ee}	2.93 ^{Cc}	1.7 ^{Fe}
	24	1.7 ^{Be}	1.7 ^{Bf}	1.7 ^{Bf}	1.7 ^{Bd}	1.7 ^{Be}
Yeasts and moulds	0	3.48 ^{Aa}	3.48 ^{Aa}	3.48 ^{Aa}	3.48 ^{Aa}	3.48 ^{Aa}
	0.5	3.22 ^{Bb}	3.16 ^{Cb}	3.06 ^{Db}	2.81 ^{Eb}	2.54 ^{Fb}
	1	3.16 ^{Bc}	2.93 ^{Dc}	2.98 ^{Cc}	2.65 ^{Ec}	1.7 ^{Fe}
	3	2.88 ^{Bd}	2.54 ^{Cd}	1.7 ^{Dd}	1.7 ^{Dd}	1.7 ^{Dc}
	5	2.81 ^{Be}	2.4 ^{Ce}	1.7 ^{Dd}	1.7 ^{Dd}	1.7 ^{Dc}
	24	2.18 ^{Bf}	1.7 ^{Cf}	1.7 ^{Cd}	1.7 ^{Cd}	1.7 ^{Cc}
Total mesophilic bacteria	0	4.48 ^{Aa}	4.48 ^{Aa}	4.48 ^{Aa}	4.48 ^{Aa}	4.48 ^{Aa}
	0.5	4.39 ^{Bb}	3.94 ^{Cb}	3.8 ^{Db}	3.7 ^{Eb}	3.55 ^{Fb}
	1	4.3 ^{Bc}	3.75 ^{Cc}	3.24 ^{Fc}	3.61 ^{Dc}	3.48 ^{Ec}
	3	4.0 ^{Bd}	3.67 ^{Cd}	3.19 ^{Ed}	3.27 ^{Dd}	2.98 ^{Fd}
	5	3.86 ^{Be}	3.19 ^{Ce}	3.13 ^{De}	3.02 ^{Ee}	2.18 ^{Fe}
	24	3.7 ^{Bf}	2.18 ^{Cf}	2.18 ^{Cf}	2.18 ^{Cf}	2.18 ^{Cc}
*Data represent average value of three counts of colonies.						
^{ABCD} means within row with different letters indicate significant difference (p<0.05) between treatments.						

^{abcd} means within column with different letters indicate significant difference ($p < 0.05$) between ozone exposure time.

Table 1: Effect of exposure time and ozone concentration on microorganism count on ‘Sekaki’ papaya fruit (log cfu/g).

Yeast and mould counts

The initial yeast and mould count was 3.48 log cfu/g. It decreased significantly as ozone concentration increased. One hour of ozone at 0.05, 0.5, 2.0, 3.5 and 5.8 ppm reduced the yeast and mould count to 3.16, 2.93, 2.98, 2.65 and 1.7 log cfu/g, respectively (Table 1). A decrease in yeast and mould count with longer ozone treatment on dried figs has been reported by Oztekin et al. (2006).

The moulds commonly associated with the spoilage of fruits and vegetables include *Botrytis cinerea* and *Aspergillus niger*. They produce a cocktail of enzymes such as hydrolases that are able to break down cell walls of produce, causing spoilage (Walton, 1994). The anthracnose symptom which commonly occurs in papaya is caused by the fungus *Colletotrichum gloeosporioides*. The effectiveness of ozone treatment on fungus *C. gloeosporioides* has been reported by Onget et al. (2012). The major factor determining spore resistance to biocidal agents appears to be the spore coat and ozone treatment may cause damage to the surface layer as well as to the outer and inner coats of fungus spores. Such damage facilitates the action of ozone on the cortex, and finally causes spore inactivation through intracellular damage (Kim et al., 2003; Young and Setlow, 2004).

To reduce yeast and mould activity, ozone should be applied either for long periods at low concentration, or conversely for short periods at higher concentrations (Najafi and Khodaparast, 2008). At 5.8 ppm, no yeasts and moulds were found after 1 h of exposure. Likewise, no yeasts and moulds were seen in fruit samples exposed for 3 h to 2.0, 3.5 and 5.8 ppm. Some yeasts are very effective at fermenting single sugars to produce alcohol and other volatiles that affect fruit quality (Barnett et al., 2000). The destruction of yeasts and moulds just after harvesting reduces the possibility of aflatoxin formation before the next processing steps (Ahmed and Ahmed, 1997; Ahmed and Robinson, 1999), which is a better preventive strategy than a subsequent complicated detoxification process.

Total coliform counts

The initial mean value of coliform bacteria was 4.13 log cfu/g (Table 1). After 5 h of ozone at 0.05, 0.5, 2.0, 3.5 and 5.8 ppm, coliform counts were reduced to 2.98, 2.65, 2.18, 2.93 and 1.7 log cfu/g, respectively. In addition, there was no increase in coliform bacteria after 24 h at all concentrations of ozone. Similarly, Achen and Yousef (2001) reported that the use of ozone-containing water for washing apples decreased the counts of *E. coli* O157:H7.

Physical quality parameters

Apart from food safety, it is necessary to investigate the quality of fruit is not negatively affected after ozone application. There were significant differences in weight loss between treated and untreated fruits (Table 2). The percentage of weight loss was smaller in all the treated fruits (24.10-25.32 %) compared with the untreated controls (25.47 %) at day 14. In this study, it showed that treated fruit maintained the full typical colour and reduced weight loss. Rodoniet al. (2010) also found reduced weight loss for ozone-exposed tomatoes after 9 days storage at 20 °C. Papaya fruit treated with ozone were also significantly firmer than the untreated fruit (Table 2). Fruit treated with 2 ppm ozone were the most firm (16.43 N) at day 14, when compared to other treated fruit (10.87-14.32 N). This result was in agreement with the findings of Aguayo et al. (2006) who reported that ozone treatments reduced softness in whole tomato fruit. Other authors have also described that ozone exposure resulted in better firmness retention (Tzortzakiset al., 2007; Rodoniet al., 2010). However, the visual quality of the fruit in terms of values of lightness (L^*), hue angle (h°) and chroma (C^*) was maintained after ozone treatments over the storage period and were not significantly different from untreated samples (Table 2).

Physical quality parameters	Storage period (days)	Ozone concentration (ppm)					
		0	0.05	0.5	2	3.5	5.8
Weight Loss (%)	0	0 ^a	0 ^a	0 ^a	0 ^a	0 ^a	0 ^a
	2	1.87 ^a	1.76 ^a	1.02 ^b	0.94 ^b	0.56 ^c	0.61 ^c
	4	3.53 ^a	2.22 ^b	1.86 ^b	1.34 ^c	1.29 ^c	1.38 ^c
	6	6.32 ^a	5.88 ^b	4.35 ^c	3.82 ^d	4.37 ^c	4.30 ^c
	8	9.11 ^a	8.24 ^b	8.83 ^b	6.46 ^d	7.55 ^c	7.19 ^c
	10	12.95 ^a	12.11 ^b	10.73 ^c	11.05 ^c	12.15 ^b	11.12 ^c
	12	18.16 ^a	17.65 ^b	15.25 ^c	15.37 ^c	18.21 ^a	17.39 ^b
	14	25.47 ^a	25.32 ^{ab}	25.03 ^{ab}	24.51 ^{ab}	24.10 ^b	24.72 ^{ab}
Firmness (N)	0	95.04 ^d	95.08 ^d	96.28 ^c	97.32 ^b	98.07 ^a	96.75 ^c
	2	67.74 ^d	73.14 ^c	80.76 ^b	82.33 ^a	82.83 ^a	81.64 ^b
	4	24.71 ^e	38.03 ^c	40.54 ^b	31.32 ^d	42.80 ^a	41.87 ^a
	6	18.83 ^c	19.41 ^{bc}	19.34 ^{bc}	22.30 ^a	20.24 ^b	19.32 ^{bc}
	8	16.44 ^d	16.63 ^d	17.18 ^c	22.11 ^a	19.64 ^b	16.45 ^d
	10	13.40 ^d	14.64 ^{cd}	15.01 ^c	20.12 ^a	18.76 ^b	13.68 ^d
	12	11.25 ^c	12.47 ^{bc}	13.47 ^b	18.36 ^a	18.22 ^a	12.35 ^{bc}
	14	10.87 ^d	11.13 ^{cd}	12.41 ^c	16.43 ^a	14.32 ^b	11.36 ^{cd}
Lightness (L*)	0	43.26 ^a	42.36 ^{ab}	42.29 ^{ab}	41.58 ^b	40.76 ^b	44.52 ^a
	2	55.57 ^a	54.77 ^a	54.66 ^a	52.86 ^b	52.21 ^b	52.34 ^b
	4	58.52 ^a	58.34 ^a	59.12 ^a	57.21 ^b	57.36 ^b	56.54 ^b
	6	64.67 ^a	63.34 ^a	63.15 ^a	61.17 ^b	61.28 ^b	61.15 ^b
	8	61.78 ^a	61.72 ^a	61.17 ^a	59.23 ^b	58.32 ^b	58.38 ^b
	10	56.15 ^b	56.25 ^b	56.21 ^b	61.26 ^a	60.42 ^a	59.19 ^a
	12	50.23 ^b	52.12 ^{ab}	53.61 ^a	53.11 ^a	53.12 ^a	53.42 ^a
	14	46.21 ^{ab}	47.32 ^a	48.53 ^a	45.76 ^{ab}	47.23 ^a	43.95 ^b

Hue angle (h°)	0	122.10 ^a	121.24 ^{ab}	120.33 ^b	123.12 ^a	123.18 ^a	120.23 ^b
	2	119.29 ^a	120.25 ^a	118.15 ^b	118.26 ^b	120.53 ^a	118.46 ^b
	4	102.64 ^a	102.45 ^a	102.48 ^a	102.11 ^a	103.57 ^a	103.65 ^a
	6	96.13 ^b	96.72 ^b	96.71 ^b	98.06 ^a	99.83 ^a	98.33 ^a
	8	87.81 ^{ab}	87.93 ^{ab}	87.96 ^{ab}	87.12 ^b	88.54 ^a	88.84 ^a
	10	79.34 ^b	79.82 ^b	80.45 ^a	81.21 ^a	81.72 ^a	80.56 ^a
	12	73.93 ^b	73.54 ^b	74.42 ^a	74.57 ^a	75.01 ^a	75.41 ^a
	14	67.56 ^b	67.43 ^b	67.78 ^b	68.28 ^a	68.98 ^a	69.39 ^a
Chroma (C°)	0	25.21 ^a	26.22 ^a	25.34 ^a	25.43 ^a	25.48 ^a	26.12 ^a
	2	33.45 ^a	32.23 ^a	31.12 ^b	31.57 ^b	31.35 ^b	31.36 ^b
	4	44.83 ^a	44.67 ^a	44.33 ^a	42.65 ^b	42.87 ^b	42.39 ^b
	6	48.47 ^a	47.86 ^a	47.18 ^a	37.77 ^b	36.32 ^b	36.67 ^b
	8	52.54 ^a	51.76 ^a	52.46 ^a	48.34 ^b	48.32 ^b	47.45 ^b
	10	43.36 ^b	42.71 ^b	43.43 ^b	45.71 ^a	45.73 ^a	44.92 ^a
	12	37.82 ^a	38.53 ^a	38.23 ^a	39.54 ^a	39.41 ^a	38.43 ^a
	14	28.61 ^a	28.56 ^a	28.38 ^a	29.34 ^a	29.13 ^a	28.33 ^a
Values are means of three experiments with four replicates per experiment.							
^{abcd} means within row with different letters indicate significant difference (p<0.05) between treatments.							

Table 2: Effect of ozone concentration on physical quality parameters of ‘Sekaki’ papaya after treated for 24 h and later stored up to 14 days at ambient storage (25±3°C and 70±5% RH).

Chemical quality parameters

The soluble solids concentration (SSC) of ‘Sekaki’ papaya fruits increased over the storage period. However, a reduction in SSC was observed after 8 days and 10 days of storage, for untreated fruit and treated fruit respectively. Highest SSC was obtained from fruits treated with 2.0 ppm ozone (11.02 %) at 10 days of storage (Figure 1).

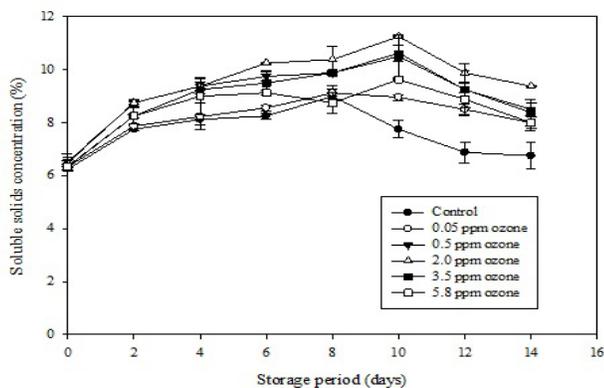


Figure 1 : Effect of ozone concentration on soluble solids concentration of ‘Sekaki’ papaya after treated for 24 h and later stored up to 14 days at ambient storage (25±3°C and 70±5% RH). Each value is the mean of four replicates ± SE.

Therefore, storage period and ozone concentration influenced the SSC changes in papaya fruit, which was consistent with the reported SSC increase in strawberry (Kuteet al., 1995) and papaya (Ali et al., 2014) fruits in response to ozone exposure. Titratable acidity (TA) of treated and untreated papaya fruit showed no difference, ranging from 0.50 to 1.57 %, and neither storage period nor ozone concentration had a significant effect on this parameter (Table 3). Similarly, no significant effect of ozone on TA was reported by Garcia et al. (1998) for navel oranges and Perez et al. (1999) for the storage of strawberry.

Chemical quality parameters	Storage period (days)	Ozone concentration (ppm)					
		0	0.05	0.5	2	3.5	5.8
Weight Loss (%)	0	1.56 ^a	1.54 ^a	1.54 ^a	1.55 ^a	1.55 ^a	1.56 ^a
	2	1.52 ^a	1.51 ^a	1.51 ^a	1.46 ^b	1.48 ^b	1.53 ^a
	4	1.36 ^b	1.38 ^b	1.36 ^b	1.40 ^a	1.41 ^a	1.42 ^a
	6	1.11 ^b	1.13 ^b	1.18 ^b	1.23 ^a	1.24 ^a	1.25 ^a
	8	0.99 ^b	1.01 ^b	1.02 ^b	1.06 ^{ab}	1.10 ^a	1.11 ^a
	10	0.76 ^a	0.76 ^a	0.75 ^a	0.72 ^b	0.73 ^b	0.75 ^a
	12	0.67 ^a	0.66 ^a	0.66 ^a	0.63 ^b	0.64 ^b	0.66 ^a
	14	0.52 ^a	0.51 ^a	0.51 ^a	0.50 ^a	0.51 ^a	0.52 ^a
	Values are means of three experiments with four replicates per experiment.						
^{abcd} means within row with different letters indicate significant difference (p<0.05) between treatments.							

Table 3: Effect of ozone concentration on titratable acidity of ‘Sekaki’ papaya after treated for 24 h and later stored up to 14 days at ambient storage (25±3°C and 70±5% RH).

Conclusions

Treatment of papaya fruit with gaseous ozone reduced significantly the total count of mesophilic microorganisms, coliform bacteria, yeasts and moulds. Significant differences between exposure times were found for all treatments. It can be concluded that a minimum of 5 h of ozone treatment at 5.8 ppm could be successfully used for eliminating the coliforms, total mesophilic bacteria, yeasts and moulds. However, longer exposure times (up to 24 h) were required to reduce total counts of mesophilic bacteria, coliform bacteria, yeasts and moulds for all the ozone concentrations tested. Fumigation by ozone reduced the risk of microbial spoilage and maintained acceptable physical and chemical qualities of the fruit during 14 days of ambient storage.

Acknowledgements

We are grateful to MedKlinn International Sdn. Bhd. for providing financial and technical support for our research.

References

1. Achen M and Yousef AE (2001) Efficacy of ozone against *Escherichia coli* O157:H7 on apples. *J. Food Sci.* 66: 1380-1384.
2. Aguayo E, Escalona VH, Artès F (2006) Effect of cyclic exposure to ozone gas on physicochemical, sensorial and microbial quality of whole and sliced tomatoes. *Postharvest Biol. Technol.* 39: 169-177.
3. Ahmed I and Ahmed A., (1997) Susceptibility of date fruits (*Phoenix dactylifera*) to aflatoxin production. *J. Sci. Food Agric.* 74: 64-68.
4. Ahmed I and Robinson RK (1999) The ability of date extracts to support the production of aflatoxins. *Food Chem.* 60: 307-312.
5. Ali A1, Ong MK, Forney CF (2014) Effect of ozone pre-conditioning on quality and antioxidant capacity of papaya fruit during ambient storage. *Food Chem* 142: 19-26.
6. APHA 1992. *Compendium of Methods for the Microbiological Examination of Foods* (16th ed., 734). Speck, M. L. (Ed.). American Public Health Association, Washington.
7. Barnett JA, Payne RW, Yarrow D (2000) *Yeasts: Characteristics and Identifications* (3rd ed.). Cambridge University Press, Cambridge, UK, pp. 395-401.
8. Beuchat LR (1992) Surface disinfection of raw produce. *Dairy, Food Environ. Sanit.* 12, 6-9.
9. Chen J (2002) Microbial enzymes associated with fresh-cut produce, in: Lamikanra, O. (Ed.), *Fresh-cut Fruits and Vegetables: Science, Technology and Market*. CRC Press, Boca Raton, FL, pp. 249-266.
10. Eckert JW and Ogawa JM (1988). The chemical control of postharvest diseases: deciduous fruits, berries, vegetables and root/tuber crops. *Annu. Rev. Phytopathol.* 26: 433-469.
11. Garcia JM, Castellano JM, Nadas A, Olias JM (1998) Effect of ozone on citrus cold storage. *COST*, v. 1. pp. 237-241.
12. Guzel-Seydim Z, Greene AK, Seydim AC (2004). Use of ozone in the food industry. *Lebensm.-Wiss.u.-Technol.* 37: 453-460.
13. Hewajulige, I.G.N., Sultanbawa, Y., Wijeratnam, R.S.W., Wijesundara, R.L.C., (2009). Mode of action of chitosan coating on anthracnose disease control in papaya. *Phytoparasitica* 37: 437-444.
14. Hwang ES1, Cash JN, Zabik MJ (2001) Postharvest treatments for the reduction of mancozeb in fresh apples. *J Agric Food Chem* 49: 3127-3132.
15. Kells SA1, Mason LJ, Maier DE, Woloshuk CP (2001) Efficacy and fumigation characteristics of ozone in stored maize. *J Stored Prod Res* 37: 371-382.
16. Kim JG and Yousef AE (2000) Inactivation kinetics of foodborne spoilage and pathogenic bacteria by ozone. *J. Food Sci.* 65: 521-528.
17. Kim JG1, Yousef AE, Khadre MA (2003) Ozone and its current and future application in the food industry. *Adv Food Nutr Res* 45: 167-218.
18. Kute KM, Zhou C, Barth MM (1995) The effect of ozone exposure on total ascorbic acid activity and soluble solids contents in strawberry tissue. In *Proceedings of the Annual Meeting of the Institute of Food Technologists (IFT)*, New Orleans, LA, USA, January 28-February 2, p. 28.
19. Maqbool M, Ali A, Alderson, PG, Mohamed MTM, Siddiqui Y, et al. (2011). Postharvest application of gum arabic and essential oils for controlling anthracnose and quality of banana and papaya during cold storage. *Postharvest Biol. Technol.* 62: 71-76.
20. Mendez F, Maier DE, Mason LJ, Woloshuk CP, (2002) Penetration of ozone into columns of stored grains and effects of chemical composition and processing performance. *J. Stored Prod. Res.* 39: 33-34.
21. Najafi MBH and Khodaparast MHH (2008) Efficacy of ozone to reduce microbial populations in date fruits. *Food Control* 20: 27-30.
22. Nguyen-the C1 and Carlin F (1994) The microbiology of minimally processed fresh fruits and vegetables. *Crit Rev Food SciNutr* 34: 371-401.
23. Ong KC, Cash JN, Zabik MJ, Siddiq M, Jones AL (1996) Chlorine and ozone washes for pesticide removal from apples and processed apple sauce. *Food Chem.* 55: 153-160.
24. Ong MK, Kazi FK, Forney CF, Ali A (2012) Effect of gaseous ozone on papaya anthracnose. *Food Bioprocess Technol.* DOI: 10.1007/s11947-012-1013-4.
25. Ozkan R, Smilanick JL, Karabulut OA, (2011). Toxicity of ozone gas to conidia of *Penicillium digitatum*, *Penicillium italicum* and *Botrytis cinerea* and control of gray mold on table grapes. *Postharvest Biol. Technol.* 60: 47-51.
26. Oztekin S, Zorlugenc B, Zorlugenc FK, (2006) Effects of ozone treatment on microflora of dried figs. *J. Food Eng.* 75: 396-399.
27. Palou L, Crisosto CH, Smilanick JL, Adaskaveg JE, Zoffoli JP (2002). Effects of continuous 0.3 ppm ozone exposure on decay development and physiological responses of peaches and table grapes in cold storage. *Postharvest Biol. Technol.* 24: 39-48.
28. Pérez AG1, Sanz C, Ríos JJ, Ollás R, Ollás JM (1999) Effects of ozone treatment on postharvest strawberry quality. *J Agric Food Chem* 47: 1652-1656.
29. Ranganna S (1999) *Handbook of Analysis and Quality Control for Fruit and Vegetable Products*. (2nd ed.) Tata McGraw-Hill, New Delhi, pp. 1-29, 163, 164, 578-582, 881-966.
30. Rodoni L1, Casadei N, Concellón A, Chaves Alicia AR, Vicente AR (2010) Effect of short-term ozone treatments on tomato (*Solanum lycopersicum* L.) fruit quality and cell wall degradation. *J Agric Food Chem* 58: 594-599.
31. Rouse S1, Harnett D, Vaughan A, van Sinderen D (2008) Lactic acid bacteria with potential to eliminate fungal spoilage in foods. *J Appl Microbiol* 104: 915-923.
32. Sharma RR1, Demirci A, Beuchat LR, Fett WF (2002) Inactivation of *Escherichia coli* O157:H7 on inoculated alfalfa seeds with ozonated water and heat treatment. *J Food Prot* 65: 447-451.
33. Spadaro D, Frati S, Garibaldi A, Gullino ML (2008) Efficacy of biocontrol yeasts against *Penicillium expansum* and *Patulin* in different cultivars of apple in postharvest. Paper presented at 16th IFOAM Organic World Congress, Modena, Italy, June 16-20, 2008. Available at <http://orgprints.org/view/projects/conference.html>.
34. Toivonen PMA and DeEll JR (2002) Physiology of fresh-cut fruits and vegetables, in: Lamikanra, O. (Ed.), *Fresh-cut Fruits and Vegetables: Science, Technology and Market*. CRC Press, Boca Raton, FL, pp. 91-123.
35. Tzortzakis N, Borland A, Singleton I, Barnes J (2007) Impact of atmospheric ozone-enrichment on quality-related attributes of tomato fruit. *Postharvest Biol. Technol.* 45: 317-325.
36. Tzortzakis NG, Singleton I, Barnes JD (2008) Impact of low-level atmospheric ozone-enrichment on black spot and anthracnose rot of tomato fruit. *Postharvest Biol. Technol.* 47: 1-9.
37. Ukuku DO1, Fan X, Kozempel MF (2006) Effect of vacuum-steam-

- vacuum treatment on microbial quality of whole and fresh-cut cantaloupe. *J Food Prot* 69: 1623-1629.
38. United States Food and Drug Administration (US-FDA), 1997. Substances generally recognized as safe, proposed rule. *Fed. Regist.* 62, 18937–18964 (April 19, 1997).
39. Walton JD1 (1994) Deconstructing the Cell Wall. *Plant Physiol* 104: 1113-1118.
40. Wang CY, Wang SY, Yin JJ, Parry J, Yu LL, (2007) Enhancing antioxidant, antiproliferation and free radical scavenging activities in strawberries with essential oils. *J. Agric. Food Chem.* 55: 6527-6532.
41. Watada AE, Ko NP, Minott DA (1996) Factors affecting quality of fresh-cut horticultural products. *Postharvest Biol. Technol.* 9: 115-125.
42. Xu and Liangji (1999) Use of ozone to improve the safety of fresh fruits and vegetables. *Food Technol.* 53(10), 58 -62.
43. Young SB1 and Setlow P (2004) Mechanisms of *Bacillus subtilis* spore resistance to and killing by aqueous ozone. *J Appl Microbiol* 96: 1133-1142.
44. Zhuang H, Barth MM, Hankinson TR (2003) Microbial safety, quality and sensory aspects of fresh-cut fruits and vegetables, in: Novak, J. S., Sapers, G. M. and Juneja, V. K. (Eds.), *Microbial Safety of Minimally Processed Foods*. CRC Press, Boca Raton, FL, pp. 255-278.