

Effect of gaseous ozone and hot water on microbial and sensory quality of cantaloupe and potential transference of *Escherichia coli* O157:H7 during cutting

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Abstract

The effect of gaseous ozone and hot water, alone or in combination, on the sensory and microbial quality of cantaloupe melon was investigated. *Escherichia coli* O157:H7 transmission from the rind to edible melon flesh during cutting practices was also investigated. Four different treatments consisting of hot water (75 °C, 1 min), gaseous ozone (10,000 ppm, 30 min), gaseous ozone supplied by carbon monoxide gas and the combination of hot water and gaseous ozone were evaluated. Sensory quality and growth evolution of aerobic mesophilic and psychrotrophic bacteria, coliforms and molds were studied. In general, hot water, gaseous ozone, and the combination of hot water and gaseous ozone were effective in reducing total microbial population. The combination of hot water and gaseous ozone was the most effective treatment to control microbial growth achieving 3.8, 5.1, 2.2 and 2.3 log reductions for mesophilic and psychrotrophic bacteria, molds and coliforms, respectively. However no significant differences were observed between gaseous ozone and gaseous ozone supplied by with carbon monoxide gas. There was no evidence of damage in melons treated with hot water, ozone or their combination and they maintained initial texture and aroma. Therefore, the combination of hot water and gaseous ozone may be an efficient and promising treatment for controlling microbial growth and maintaining sensory quality of melons.

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1. Introduction

Despite the commensal status of the majority of *Escherichia coli* strains, pathogenic strains, particularly enterohaemorrhagic *E. coli* O157:H7, is recognized as an important emerging foodborne pathogen. Fresh fruits such as cantaloupes and other melons have been identified as the vehicle for *E. coli* O157:H7 outbreaks (Uchimura et al., 1998). Cantaloupe has been associated with other foodborne pathogens such as Norovirus (Iversen et al., 1987) and

different serovars of *Salmonella enterica* (CDC and Centers for Disease Control and Prevention, 1991; FDA and Food and drug Administration, 1991; CDHS and California Department of Health Services, 2000, 2001, 2002;). Melons can be contaminated through inadvertent contact with raw meat (Harris et al., 2003) or human handlers (Iversen et al., 1987). In other cases the contamination was thought to have been soil on the melon rind (Mohle-Boetani et al., 1999), packing house wash water, or shipping ice (Hedberg et al., 1994; Tauxe, 1997). Cantaloupe can become contaminated during growth, postharvest handling and packaging, transportation, distribution or during final preparation at food service or in the home (Beuchat, 1996).

Transfer of pathogens such as *Salmonella*, from the rind to edible cantaloupe flesh can occur during cutting (Suslow et al., 2000; Ukuku and Sapers, 2001). Some studies have

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been done to determine the efficacy of washing and sanitizing in order to prevent spoilage microorganism and pathogen development in cantaloupe and watermelon (Park and Beuchat, 1999). Agents that are chlorine based have been often used to sanitize produce and surfaces, as well as reduce microbial population in water applied during cleaning and packing operations (Delaquis et al., 2004). However, the production of chlorinated organic compounds, such as trihalomethanes, which are potential carcinogens (Fawell, 2000) or other disinfection-by-products has created the need to investigate the efficacy of non-traditional sanitizers and other alternative technologies. Therefore, quality assuring or extending preservation methods for fresh-cut fruit and vegetable are still under study. The concept of using multiple intervention methods or various preservation technologies that are healthier for consumers is increasing in their importance (Parish and Davidson, 1993; Soliva-Fortuny and Martín-Belloso, 2003; Beltrán et al., 2005).

In the last years, some studies have been conducted about the use of hot water as an alternative to melon disinfection (Fallik et al., 2000; Annous et al., 2004; Ukuku et al., 2004; Fan et al., 2006; Solomon et al., 2006). The results of these studies showed the efficacy of the hot water alone (Fallik et al., 2000; Solomon et al., 2006) or in combination with hydrogen peroxide (Ukuku et al., 2004) and low dose irradiation (Fan et al., 2006) to reduce microbial load on contaminated whole melons. Ozone has been extensively applied to drinking water for sanitation purposes as an important efficacy against bacteria, molds, viruses and protozoa (Korich et al., 1990; Restaino et al., 1995). The decrease of pathogens including *Salmonella typhimurium*, *Yersinia enterocolitica*, *Staphylococcus aureus*, *Listeria monocytogenes* and *E. coli* O157:H7 has also been described (Restaino et al., 1995; Singh et al., 2002; Selma et al., 2006). Therefore, the use of ozonated water has been suggested as an interesting alternative to traditional sanitizers due to its efficacy at low concentrations and short contact time as well as the breakdown to non-toxic products (Graham 1997; Rice, 1999). The use of O₃ gas could be another alternative to reduce microbial populations on the product prior to cutting. Modifying atmospheres to contained controlled levels of O₃ during the storage of grapes and apples has resulted in effectively reducing fungal growth and increasing the shelf-life of these fruits (Bazarova 1982; Sarig et al., 1996). However, due to its strong oxidizing activity, O₃ may also cause physiological injury to produce (Parish et al., 2003). Therefore, the possible negative impact of O₃ treatment on fruit and vegetable sensory quality (Fanchun and Wang, 2003) warrants further study.

The objective of this study was to investigate the effect of hot water and gaseous O₃ alone or in combination, on the microbial and sensory quality of cantaloupes. We also investigated *E. coli* O157:H7 transference from the rind to edible melon flesh during cutting practices.

2. Materials and methods

2.1. Culture preparation

The bacterial strain used in this study was *E. coli* O157:H7 LJH537 (associated with *E. coli* outbreak in lettuce and obtained from Dr. Linda Harris, Department of Food Science and Technology, University of California). The culture of *E. coli* O157:H7 was transformed to express the green fluorescent protein (GFP) on a stable plasmid as described by O'Callaghan and Charbit (1990). GFP plasmid (Joyner and Lindow, 2000) was isolated from *E. coli* TVS 297 (GFP expressing and kanamycin-resistant, obtained from Dr. Trevor Suslow, Department of Vegetable Crops, University of California) using Wizard Plus minipreps DNA purification System (Promega, USA). The GFP emits green light when is excited with ultraviolet (UV) radiation and no substrate or cofactor is required for fluorescence. *E. coli* O157:H7 was transformed by electroporation with the GFP plasmid as described by O'Callaghan and Charbit (1990). Transformed bacteria were selected on tryptone soy agar (TSA, Difco, Detroit, MI, USA) with kanamycin ($5 \times 10^{-2} \text{ g L}^{-1}$) (Sigma Chemical, St. Louis, MO, USA, TSA-Kan). Colonies were screened under UV-light and by PCR using primers specific for *Stx*-encoding genes *stx1* and *stx2* as described previously (Fode-Vaughan et al., 2003). Amplification products were determined by electrophoresis using 1.5% agarose gels. Transformed bacteria (*E. coli* O157:H7/GFP) were inoculated onto separate TSA-Kan plates incubated for 24 h at 37 °C. Stock bacterial cultures were kept at -70 °C in tryptone soy broth (TSB; Difco) with kanamycin ($5 \times 10^{-2} \text{ g L}^{-1}$) (TSB-Kan) containing 10% (vol/vol) glycerol (Sigma Chemical, St. Louis, MO.) and subcultured twice in TSB-Kan at 37 °C for 24 h before use.

2.2. Plant material

Unwaxed cantaloupe melons (*Cucumis melo* var. *reticulatus*) (14.0 ± 1.3 cm diameter, mean weight 900 ± 80 g) were obtained from a local wholesale supplier in Sacramento (California, USA) and transported to the laboratory where those with defects (cuts, abrasions, and compression bruises) were discarded. Melons were stored at room temperature (20 °C) for 24 h before sanitizing treatments.

2.3. Gaseous ozone treatments

An PO-12 laboratory-scale system designed by PureOx (Nevada, USA), and based at the Department of Vegetable Crops (University of California, Davis) were used. The equipment consists on a 50 L stainless steel chamber, a cooling unit (to control the treatment temperature) and a bubbler assembly (to provide a gas stream). The equipment provides the supply of O₂ and CO₂ gas to feed the O₃ generator. The supply of O₂ (1 L min^{-1}) gas was

required for O₃ production. Furthermore, CO₂ supply (100 mL min⁻¹) allowed an increase of CO concentration in the treatment chamber. The chamber temperature, relative humidity, gaseous O₃ concentration, and chamber pressure was monitored from the main control panel. Gaseous O₃ concentration in the chamber was measured with an O₃ gas analyzer (model H1-SPT, IN USA Inc., Needham, MA) which allows the O₃ generator to automatically switch on and off in order to maintain the target concentration. The excess of gas was neutralized by a catalytic destruct unit.

Whole melons (six melons each) were treated for 30 min with an O₃ concentration of 10,000 ppm O₃ (11 °C, 90–95% relative humidity) with O₂ gas (O₃ treatment) and O₂ and CO₂ gas (O₃+CO₂ treatment, resulting in the creation of CO gas), respectively. All the experiments with O₃ were made in the pilot plant of the Department of Vegetable Crops (University of California, USA) following strict safety and protection policies and procedures. Dual catalytic converters assured that all O₃ was converted to oxygen before being discharged from the unit.

2.4. Hot water treatments

Whole melons were submerged in 75 °C water in a circulating water bath (21 L of water each six melons) for 1 min. Treated melons were air-dried under a biohazard safety cabinet for 15 min. Half of the melons were then treated for 30 min with 10,000 ppm O₃ (11 °C, 90–95% relative humidity).

2.5. Sample preparation and enumeration of surviving natural microflora

Melons were peeled with a fruit peeler (Peel-All Fruit Peeler, Model CP44, Muro. Corp., Tokyo, Japan). The entire peel was transferred into stomacher bags with 225 mL of 1% of buffered peptone water pH 7 and vigorously rubbed and intermittently inverted for 1 min. Aliquots were taken from each bag, serially diluted in 0.1% peptone water, and plated on various agar media. Six cantaloupes per treatment were sampled in each of three separate experiments.

Total aerobic mesophilic and psychrotrophic bacteria were enumerated by the standard plate count method using plate count agar (PCA, Difco, Detroit, MI, USA) and incubation at 30 ± 1 °C for 48 h and at 4 ± 1 °C for 7 days, respectively. Mold counts were performed in potato dextrose agar (PDA, Difco) plus 10% tartaric acid and 0.1 g L⁻¹ streptomycin (Sigma Chemical, St. Louis, MO, USA) and incubation at 25 ± 1 °C for 5–7 days. Coliforms and *E. coli* were enumerated using *E. coli* and coliform chromagar (ECC; CHROMagar™, Santa Maria, CA, USA) and incubation at 37 ± 0.5 °C for 24 h. Microbial counts were expressed as log CFU g⁻¹ of rind.

2.6. Sample preparation and sensory evaluation

After sanitizing treatments, whole melons were peeled and cut in cubes (3 × 3 cm, 30 ± 3 g) using sharp stainless steel knives. Ten fresh-cut melon cubes (300 ± 5 g) were packaged in each polypropylene container (165 × 165 × 60 mm) using passive modified atmosphere packaging (MAP) without initial gas injection. All samples were stored at 5 °C for up to 8 days and sensory quality was evaluated initially and after 4 and 8 days. Four-member expert panel evaluated three containers (30 melon cubes) per each treatment and evaluation period. Melon cubes of each container were randomly numbered. Visual quality was scored on a 9–1 scale, where 9 = excellent, 7 = good, 5 = fair, 3 = poor, 1 = unusable/unacceptable. Aroma was scored based on a scale of 1–5, where 1 = none, 2 = slight, 3 = moderate, 4 = pleasant, mild cantaloupe odor, 5 = full, characteristic cantaloupe odor. Off-odors and translucency were scored on a 1–5 scale, where 1 = none, 2 = slight, 3 = moderate, 4 = moderate/severe and 5 = severe.

2.7. Firmness, color changes and soluble solid content

Firmness of fresh-cut melon was carried out by using a TA-TX Texture Analyzer (Texture Technologies Corp. Scarsdale, New York, USA). This test was based on the resistance of the center sides of each melon piece to pressure, by a puncture test, using a round-tipped cylindrical 11 mm diameter probe (1 mm s⁻¹ to a depth of 5 mm). Firmness of all sides of melon cubes was measured. Color changes of fresh-cut melon cubes were analyzed on the pulp surface with a Minolta Chroma Meter CR 300 (Minolta Co. Ltd., Osaka, Japan). Soluble solids content (SS) was measured using a digital temperature compensated Antago N1 handheld refractometer (Tokyo, Japan).

2.8. Inoculation of cantaloupes and transfer of *E. coli* O157:H7° from the rind to the flesh during cutting

One colony of *E. coli* O157:H7/GFP was grown in 20 mL of sterile TSB-Kan until stationary phase at 37 °C. Two milliliters of culture were centrifuged for 5 min at 1800g in a microcentrifuge (model 5415 D, Brinkmann Instrument Inc., Westbury, N.Y.). The pellet was washed twice and re-suspended in 20 mL 0.1% peptone water. Three circles of 4 cm diameter were marked on each whole melon. The center of the circles were inoculated with 200 µL of 0.1% peptone water (negative control) and 200 µL of 10⁴, 10⁵, 10⁶, 10⁷, 10⁸ and 10⁹ CFU mL⁻¹ of bacteria and placed inside a biohazard safety cabinet for 1 h and stored at 5 °C for 4 days.

Inoculated and non-inoculated cantaloupes were cut into cores approximately 4 cm diameter by 1 cm thick and then cut again into two sections through the point of inoculation with a sterile knife and lastly the rind was removed. Cylinders were incubated at 37 °C for 1 h and the transference of bacteria from rind was determined by

plating on TSA-Kan plates, visualization of green fluorescence on the flesh of melon cubes under UV-light and PCR analysis. For detection on TSA-Kan plates, two methods were carried out:

- (A) Four cubes of melon were transferred into stomacher bags with 50 mL sterile buffered peptone water. Samples were homogenized with a stomacher (stomacher lab blender, model 80 Biomaster, Seward, UK) for 1 min and incubated at 37 °C for 2 h. Aliquots were taken from each bag (0.2 mL) and plated in duplicate on TSA-Kan plates with incubation at 37 °C.
- (B) Each cube of melon was placed directly on TSA-Kan plates for 1 h at 37 °C. Cubes were then removed and plates were incubated at 37 °C for 24 h.

To confirm the presence-absence of *E. coli* O157:H7 a PCR method was used. Cubes of melon were transferred into stomacher bags with 225 mL of modified tryptone soy broth supplemented with kanamycin ($5 \times 10^{-2} \text{ g L}^{-1}$) and incubated for 24 h at 37 °C. Two milliliters of post-enrichment culture were transferred to a polypropylene cap tube and washed three times with Tris-EDTA 1X solution pH 8 (Cole-Parmer Instrument Company, Illinois, USA) by centrifugation at 1800g/10 min. Pellet was resuspended in sterile nanopure water and placed in boiling water bath for 15 min. After cooling to room temperature a PCR reaction was run using primers specific for *Stx*-encoding genes *stx1* and *stx2* as described previously (Fode-Vaughan et al., 2003). Amplification products were determined by electrophoresis using 1.5% agarose gels.

2.9. Statistical analysis

There were three separated experiments per treatment. Microbial data represent the mean of the three experiments, each consisting of triplicate samples (six cantaloupes) per each treatment. Sensory data represent the mean of the three experiments with three replicates per each treatment and evaluation period. Each replicate consists of a container with ten melon cubes. Analysis of variance (ANOVA), followed by Tukey's method with a

significant level of $P \leq 0.05$ was carried out on these data using SPSS (Windows 2000, Statistical Analysis).

3. Results and discussion

3.1. Effect of sanitizing treatments on microbial stability in melon

The effects of the different sanitizing treatments on the native microflora of melon are shown in Table 1. Microbial population on the control samples (non treated melons) averaged 5.9, 5.6, 2.2 and 2.3 log CFU g^{-1} of rind for mesophilic and psychrotrophic bacteria, molds and coliforms, respectively. No *E. coli* was recovered from selected whole melons (data not shown). All sanitizing treatments reduced initial total counts of mesophilic and psychrotrophic bacteria, molds and coliforms compared to untreated samples (Table 1). Gaseous O_3 treatment (10,000 ppm/30 min) reduced total mesophilic counts by 1.1 log units. No significant differences were found between the number of microorganisms in melons treated with gaseous O_3 and gaseous O_3 supplied with CO_2 . Therefore, an additional effect of CO gas on microbial inactivation was not found despite CO gas has been described as an effective antimicrobial (Loss and Hotchkiss, 2002). The efficacy of hot water treatment (75 °C/1 min) on microbial inactivation was superior to O_3 treatments achieving 2.6 and 2.3 log reductions on mesophilic bacteria and coliforms, respectively (Table 1). The temperature of the wash water did not decrease more than 2.0 ± 0.3 °C throughout the treatment. On the other hand, the internal temperature of cantaloupe following surface pasteurization was not analyzed. However, some researchers have reported that once the cantaloupes are submerged in hot water, cantaloupe surface temperature, but not flesh temperature, increases rapidly (Annous et al., 2004). Thus, the rind insulates the flesh from extreme temperatures. Coliforms are used as indicator microorganisms of fecal contamination in water and foods and include potentially pathogen species such as *E. coli*. This microbial group was of especial concern in melon because of the high initial counts on untreated melons (2.3 log CFU g^{-1}) which were greatly

Table 1

Effect of sanitizing treatments on survival of mesophilic and psychrotrophic bacteria, molds and coliforms (log CFU g^{-1} of rind) on the surface of whole cantaloupe melons

Sanitizing treatments	Mesophilic bacteria	Psychrotrophic bacteria	Molds	Total coliforms
Untreated	5.9 a	5.6 a	2.2 a	2.3 a
10,000 ppm O_3 /30 min	4.8 b	4.3 b	0.7 b	1.0 b
10,000 ppm O_3 /30 min + CO_2	4.4 b	4.4 b	0.5 b	1.2 b
Hot water (75 °C/1 min)	3.3 c	1.3 c	0.3 c	0.0 c
Hot water (75 °C/1 min) + 10,000 ppm O_3 /30 min	2.1 d	0.5 d	0.0 d	0.0 c

^aValues represent means of three experiments with three determinations per experiment. (A–D) means within columns of the same group of microorganism followed by different letters are significantly different ($P \leq 0.05$).

reduced after this hot water treatment (75 °C/1 min). Furthermore, this hot water treatment became especially effective on psychrotrophic bacteria achieving 4.3 log reductions (Table 1). Control of these microbial groups on whole melon is important as they are the main source of contamination for fresh-cut melon and one of the main factors responsible for spoilage.

Although total mesophilic bacteria counts were not reduced by O₃ more than 1.1 log units, O₃ coupled with hot water treatment was the most effective treatment for microbial inactivation (Table 1). An additive effect was found using this combination achieving 3.8, 5.1 and 2.2 log reductions on mesophilic and psychrotrophic bacteria, and molds, respectively. This additive effect was not observed on coliforms where significant differences were not found between hot water treatment and O₃ coupled with hot water treatment. However, this important reduction on psychrotrophic bacteria has a great relevance due to the fact that whole and fresh-cut melon storage is carried out at refrigerated temperatures and this bacterial group contributes to spoilage of this product. Therefore, hot water treatment alone or combined with O₃ could be two interesting alternatives to traditional sanitizers such as sodium hypochlorite for controlling microbial counts of melons without production of potential carcinogens

3.2. Sensory evaluation of ozone and hot water treatments

Initially, the visual quality of fresh-cut melon was excellent after all sanitizing treatments and aroma, translucency, firmness were not significantly different from untreated samples (Table 2). During storage, sensory quality was maintained in all the samples and no off-flavors were developed. However, visual quality and translucency were slightly decreased and increased, respectively, after O₃ treatment combined with CO. At the end of the storage treated and untreated samples maintained the

full typical aroma, color and a very firm and turgid texture. Although Liew and Prange (1994) showed that bananas and carrots treated with O₃ gas develop black spots and have less intense color during storage, gaseous O₃ treatment did not affect the global sensory quality of fresh-cut melon after 8 day storage. Therefore, gaseous O₃ and hot water treatments alone or in combination could be interesting alternatives to inactivate pathogenic and spoilage microorganisms without considerably affecting sensory quality of fresh-cut cantaloupe melons.

3.3. Transference of *E. coli* O157:H7 from the rind to the flesh during cutting

Transference of *E. coli* O157:H7 from cantaloupe surface inoculated at different levels to fresh-cut pieces is shown in Table 3 and Fig. 1. Fresh-cut tissues prepared from non inoculated (negative control) and inoculated whole cantaloupes (3.3 log CFU/rind) were negative for *E. coli* O157:H7 by standard plating in TSA-Kan (A), direct contact of each cube of melon on TSA-Kan plates (B), UV light applied directly on the surface of melon cubes, and PCR analysis (Table 3). Fresh-cut pieces prepared from whole cantaloupes inoculated with 4.3 to 8.3 log CFU/rind were positive for the bacteria by all tested methods.

These results indicate that transfer of *E. coli* O157:H7 from the rind to the edible flesh of cantaloupe occurs and as is expected, the higher the number of bacteria on the melon rind, the more likely it is that this cross-contamination will occur. Therefore, in fresh-cut processing operations it is imperative that the melon rind be properly cleaned with sanitizing treatments before cutting. Transference of pathogenic bacteria such as *Salmonella* has also been observed during the slicing of melons (Ukuku et al., 2004) and tomatoes (Lin and Wei, 1997). *E. coli* O157:H7 or *Salmonella* can grow to high levels on cut melons stored at ambient temperature (Golden et al., 1993; Suslow, 2001). Therefore, high levels of pathogens can be reached before

Table 2
Sensory evaluation of fresh-cut melons prepared from whole cantaloupes treated with different sanitizers and stored 8 days at 5 °C

Treatment	Visual quality ^a		Aroma ^b		L		Translucency ^c		Firmness (N)		SS (%)	
	0 and 4 days	8 days	0 and 4 days	8 days	0 and 4 days	8 days	0 and 4 days	8 days	0 and 4 days	8 days	0 and 4 days	8 days
Untreated	9.0 a	9.0a	3.0a	3.5a	1.0 b	1.0b	63.9ab	64.4b	16.9ab	17.8 a	9.0 b	8.6c
10,000 ppm O ₃ /30 min	8.9 a	8.8a	3.0a	3.0b	1.2 b	1.2b	64.0ab	64.7ab	16.2 b	15.4bc	9.7 a	9.1b
10,000 ppm O ₃ /30 min + CO ₂	8.8 b	8.5b	3.0a	3.0b	1.3 a	1.5a	63.6 b	65.4ab	17.0ab	15.1c	9.7 a	9.2b
Hot water (75 °C/1 min)	9.0 a	8.9a	3.0a	3.0b	1.1 b	1.1b	64.9a	65.9a	17.9 a	16.6ab	8.6 c	8.8c
Hot water (75 °C/1 min) + 10,000 ppm O ₃ /30 min	9.0 a	8.6b	3.0a	3.0b	1.1 b	1.4a	64.0ab	62.2c	17.4ab	17.1 a	9.8 a	10.1a

Values are means of 30 replicates which were analyzed in duplicates. Data within a row followed by different letter are significantly different at ($P \leq 0.05$).

^aScale 9-1, where 9 = excellent, 7 = good, 5 = fair, 3 = poor, 1 = unusable/unacceptable.

^bScale 1-5, where 1 = none, 2 = slight, 3 = moderate, 4 = pleasant, mild cantaloupe odor, 5 = full, characteristic cantaloupe odor.

^cScale 1-5, where 1 = none, 2 = slight, 3 = moderate, 4 = moderate/severe, 5 = severe.

Table 3

Effect of external concentration of *E. coli* O157:H7 on detectable transference to cantaloupe flesh after direct cutting. (A) *E. coli* recovery by plating aliquots from stomacher bags. (B) *E. coli* detection by direct contact of each cube of melon on plates

Whole melon Inoculum level (log CFU/rind)	Fresh-cut pieces		UV Light	PCR
	Agar plates			
	A	B		
0.0±0.00	(-)	(-)	(-)	(-)
3.3±0.01	(-)	(-)	(-)	(-)
4.3±0.03	(+)	(+)	(+)	(+)
5.3±0.03	(+)	(+)	(+)	(+)
6.3±0.07	(+)	(+)	(+)	(+)
7.3±0.07	(+)	(+)	(+)	(+)
8.3±0.09	(+)	(+)	(+)	(+)

(+), positive; (-), negative.

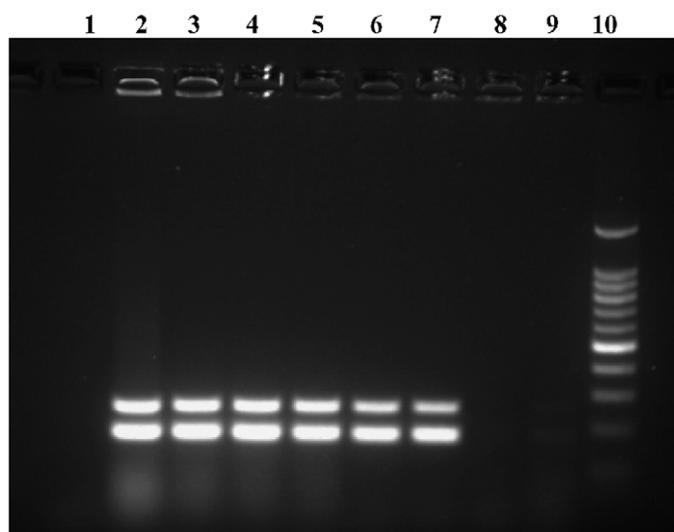


Fig. 1. *E. coli* O157:H7 PCR from melon cubes after external inoculation with different concentrations of cells. (1) negative control; (2) positive control; (3) 2×10^8 ; (4) 2×10^7 ; (5) 2×10^6 ; (6) 2×10^5 ; (7) 2×10^4 ; (8) 2×10^3 CFU/rind; (9) melon without inoculation; (10) ladder.

signs of spoilage are evident being the safety of fresh-cut produce under concern.

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