

The Effect of Ozoned Water Treatment in Preserving fresh and Extending Shelf-life of fresh Cut Melons (Cucumis Melo L.)

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Abstract: The expansion of the fresh fruit business brings both possibilities and problems for supplying high-quality fruits to the market. Furthermore, the usage of ozonated water as part of Tefa Smart Green House (SGH) strategic procedures to retain the freshness of Minimally Fresh Processed (MFP) fruit was required; also, research into pesticide contamination residues has not been widely conducted. This study used a portable ozonation apparatus (Leka OZ3000) that has been adapted to create an ozone solution of 1.6 ppm (O1), 0.8 ppm (O2), and 0.4 ppm (O3) and was utilized on Tefa SGH Polije melon slices with ozone contact durations of 10 minutes (T1) and 20 minutes (T2), respectively. Moreover, the fruit was kept at room temperature in polypropylene packaging for 3 days (S1) and 5 days (S2). For the pesticide treatment, the melons were previously prepped with 3 grams of Dithane in 1 liter of water in the form of spraying. Pesticide residues were included in the analysis (HPLC method). The findings indicated an 80% Mankozeb residual of 0.5875 mg/kg (S1), whereas using ozone at 1.6 ppm (T2) revealed an 80% Mankozeb residue of 0.1682 mg/kg (S1).

Index Terms: Minimally Fresh Processed, Melon, Ozonated Water, HPLC

I. INTRODUCTION

Melon (*Cucumis melo* L.) is the most important fruit, accounting for the fourth largest market share of fresh fruit worldwide [1]. Melon fruit is a sweet and juicy fruit that is well-known for its nutritional and therapeutic benefits. Fatty acids, polyphenols, and carotenoids are BCs found in melon that have a variety of therapeutic effects that can enhance human health [2]. Melon is a popular tropical fruit because it has an appealing appearance with yellowish-orange fruit flesh and a sweet flavor (sugar content: 12 - 13 ° brix), the texture of crispy fruit meat, a fragrant perfume, weighs around 2-3.8 kg, and has a shelf life of 10 days after harvest [3]. Melons can also be available at traditional markets and supermarkets. State Polytechnic of Jember (Polije) has begun growing melons with a standard brix rate (13°brix) in the Tefa SGH (Smart Green House). There is now an upsurge in demand for Polije goods, both for internal purposes (Civitas Academica Polije) and to service the local market. Fresh fruit consumption can be supplied by minimally fresh processed (MFP) meals or freshly cut fruits and vegetables, which are now in high demand. In addition to convenience, consumers value MFP goods for their affordability, cost-effectiveness, and quality [1][4]. Consumer demand for MFP foods, particularly fruits and vegetables, is expanding dramatically [5]. Fruits, but in the other hand, are agricultural commodities having unique properties that are easily injured and have a short shelf life [6]. As a result, effective technologies, such as agricultural system management and sanitization procedures, are required to assure extended shelf-life and fruit quality. The lack of pesticide residues that are harmful to health is driving the current demand for organic food [7].

To reduce losses and deliver fresh processed or cut fruits and vegetables of safe and good quality, the industry must use improved techniques [8]. Ozone is a vigorous oxidant that has been approved as a food sanitizer, mostly in organic farming, because it decomposes safely and spontaneously without producing harmful residues[9]. Some items used to sanitize vegetable foods, such as chlorine gas, sodium or calcium hypochlorite, and organic chlorine, should be avoided since they produce carcinogenic chlorinated compounds, such as trihalomethanes, which are harmful to human health and the environment[10]. In this context, ozonated water has emerged as a safe method of sanitization in a wide range of plant products, such as table olives[11], *Vitis vinifera* and berry [12], grape [13], papaya [10], vineyard [14], citrus fruit, stone fruit, broccoli [15] and table grapes[16].

Various studies have found that exposing some fruits and vegetables to ozone enhances their total phenol content [17][18][10] as well as other compounds with antioxidant properties [15]. The process of washing pollutants is a key stage that influences customer approval of items (both microbiological and chemical, pesticide residues). Since 1997, the European Union has prohibited the use of chlorine solution as an antibacterial ingredient in the washing process. According to Wibisono, damage to fresh fruit goods in Jember Regency ranged from 12 to 15% owing to physiological and microbiological activity, with an estimated total loss of 8 – 11% of sales revenues [19].

According to the preceding description, ozone is an antibacterial and anti-residue substance that has the potential to be developed as a substitute for chlorine in the washing process, but the aspect of ozonolysis in fresh-cut fruit has not been extensively explored.

As a result, it is important to build a fresh fruit washing installation model that combines ozone (ozonated water) with modified atmospheric packing in order to enhance the shelf life of fresh-cut melons.

II. MATERIAL AND METHODS

2.1. Materials and tools

The materials utilized are melons collected from Tefa Smart Greenhouse Polije. The chemicals used for analysis are Mankozeb (purity $\geq 99.9\%$), acetoneiril (gradient grade $\geq 99.9\%$) merck brand, ethylacetat, Na₂SO₄ anhydrate, aquabidest, and chemicals for microbiology with 16 S rRNA, including agarose, buffer tae 1x, ethidium bromide, and Gene Ruler 1 kb DNA ladder from fermented and materials for elektrofores.

The equipment utilized is a set of ozonated water system washers branded Leka modified; HPLC Agilent 1260 infinite binary LC and Zorbax Eclipse Plus C18 column; UV-Vis spectrophotometer Perlin Elmer; rotavapor; and sonikatorbmerk. Tomy Seiko Co, LTD; analytical balance sheet; vacuum filter with porous sieve 0.4 – 0.45 m and sequencing equipment including DNA Thermal Cycle brand Perkin Elmer and Takara; ultracentrifuge brand Medfriger; QIAmp Mini Spin column; ABI PRISM 3130 brand Genetic Analyzer sequencer machine complete with sequencing set; bio rad brand microcentrifuge; and fermentation equipment includes biohazard cabinet; hemocytometer; and software including Gel Analyzer 2010; Bioedite; Clustal X and Treeview.

2.2. Research Design

This study used a portable ozonation device (Leka OZ3000) modified to produce an ozone solution of 1.6 ppm (O1), 0.8 ppm (O2), and 0.4 ppm (O3) and used in Tefa SGH Polije melons with ozone contact times of 10 minutes (T1) and 20 minutes (T2) and fruit stored at room temperature for 3 days (S1) and 5 days (S2) with polypropylene packaging. Melon was previously prepared in the form of spraying with 3 grams of Dithane in 1 liter of water to condition pesticide treatment. Pesticide residues (HPLC technique), pH, discolouration, and microbial identification using 16S rRNA were all tested on items with the lowest pesticide residues.

2.3. Analysis of Pesticide Residues

The procedure for determining pesticide residue levels:

a. Preparation and Extraction of Samples

Samples of fresh cut fruit that has been diced and weighed up to 500 g before being placed in a blender. For 2-3 minutes, add 300 ml of ethyl acetate to the blender. The example is filtered through the separator gourd with glass fibre. A layer of water is removed, and 30 g of anhydrous sulfate is added to an organic layer. An evaporator with a capacity of up to 5 ml is used to compress the sample extract.

b. Cleaning

The chromatography column is filled with activated florisil (which has been heated for 2 hours at 105°C). The bottom of the column is filled with glass wool, and the florisil column is moistened by skipping 40-50 ml of hexane:acetone (4:1, v/v) mixture. Following that, the diap column is used as an example. The calculated example extract is passed through a column containing florisil and then diluted with the solvent hexane:acetone (4:1, v/v). Eluat is stored on a pumpkin and pressed using an evaporator until it is dry.

c. Analysis of Residual Levels

The dried residue is re-dissolved in 4 ml of methanol-water (1:1, v/v) and injected into the Agilent 1260 infinite binary LC device with the Zorbax Eclipse Plus C18 column, with the car phased in testing/set to the most optimum settings. A total of two liters of sample were injected into the injector, and the area of the chromatogram generated was measured.

2.4. Microbial Analysis

Selected colonies of melons were withdrawn from the MRS medium and placed in a 1.5 ml tube while mashed, with destilated water added (DW). Supernatannya was removed during centrifugation at 12,000 rpm. 200 l InstaGene Matrix was added to the pellet-containing tube, which was inculated at 56°C for 30 minutes, vortexed at high speed for 10 seconds, inculated again at 100°C for 10 minutes, vortexed again for 10 seconds, and centrifuged at 12,000 rpm for 3 minutes. The following is the next step:

- A total of 560 μ l of the AVL buffer containing the carrier RNA was piped into a 1.5 ml microcenterent tube.
- 140 μ l supernatant was added to the microcentrifuge tube above and vortex for 15 seconds. Homogenization is necessary for lysis efficiency.
- Incubation at a temperature of 15-25°C for 10 minutes. The process of lysis of viral particles will be completed in about 10 minutes, if given a longer time will have no effect on the results or quality of RNA. Virulent agents carrying the infection will be activated by administering an AVL buffer.
- A total of 560 μ l of ethanol (96 – 100%) was added to the sample and vortexed for 15 seconds.
- 5 Carefully, 630 μ l of the solution is added from the previous step to the QIAamp Mini Spin column (in 2 ml of collection tube) without wetting the surrounding edges. The tube is closed and centrifuged at 6000xg (8000 rpm) for 1 minute

- f. QIAamp mini spin columns are opened and added with 500 µl of the AW1 buffer. The plug is closed and centrifuged at 6000xg (8000 rpm) for 1 minute. The QIAamp Mini Spin column is placed on a 2 ml collection tube, and the filtrate contained in the tube is discarded. The use of AW1 buffer does not need to increase the amount of volume if the volume of solution (sample) is greater, in contrast to the AVL buffer whose volume must be given proportional to the solution (sample) given.
- g. The QIAamp Mini Spin column is opened and added with 500 µl of the AW2 buffer. The plug is closed and centrifuged at full speed 20000xg (14000 rpm) for 3 minutes.
- h. QiAamp Mini Spin column is placed on a 1.5 ml microcentrifuge tube. Tubes containing filtrate are discarded and carefully open the QIAamp Spin column to be added with 60 µl of the AVE buffer at room temperature conditions.

III. RESULTS AND DISCUSSION

3.1. Mankozeb Optimization Using HPLC

Figure 1 shows the results of Mankozeb optimization using HPLC, which reveal three chlorpyrifos standard solution absorption areas, = 820 nm, = 265 nm, and = 289 nm. Because there are no overlaps on these wavelengths, 256nm was applied in this research.

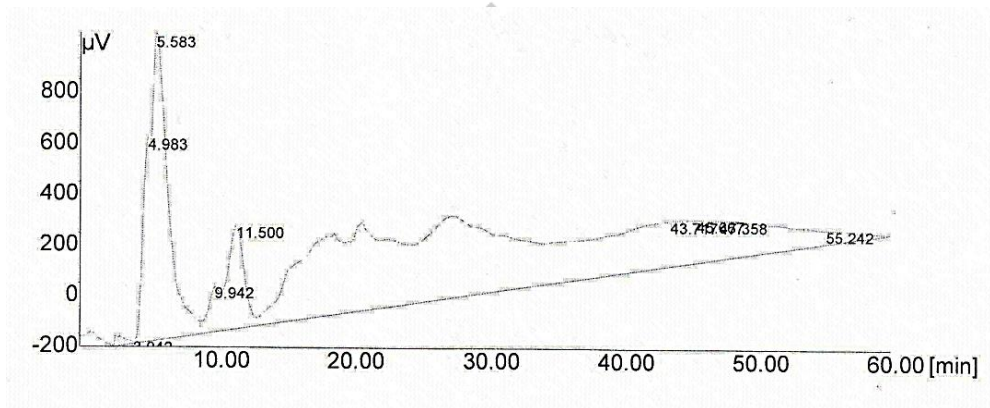


Fig. 1 Mankozeb absorption wavelengths

These wavelengths were then analyzed to optimize the HPLC measurement parameters using a DAD detector with mobile phase water and acetonitrile, which is more polar than C18. The result reveals that the composition ratio of acetonitrile:water is 50:50 (polarity index), as shown in figure 2. Meanwhile, table 1 demonstrates the relation of acetonitrile:water composition to retention duration and area.

Table 1. Result of acetonitrile: water composition to retention time and area

Acetonitril:water composition	Retention Time (minutes)	Area (mAU*s)
50:50	5.367	1028.47284
40:60	8.472	109.34973
30:70	6.374	89.49204
20:80	3.294	45.49274

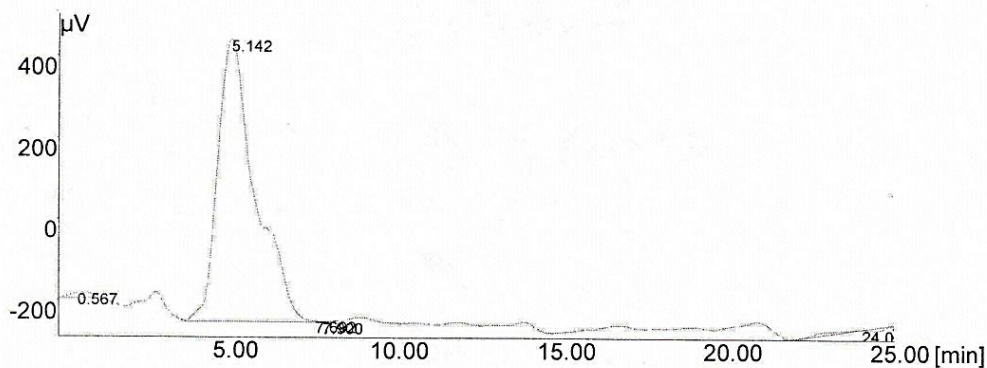


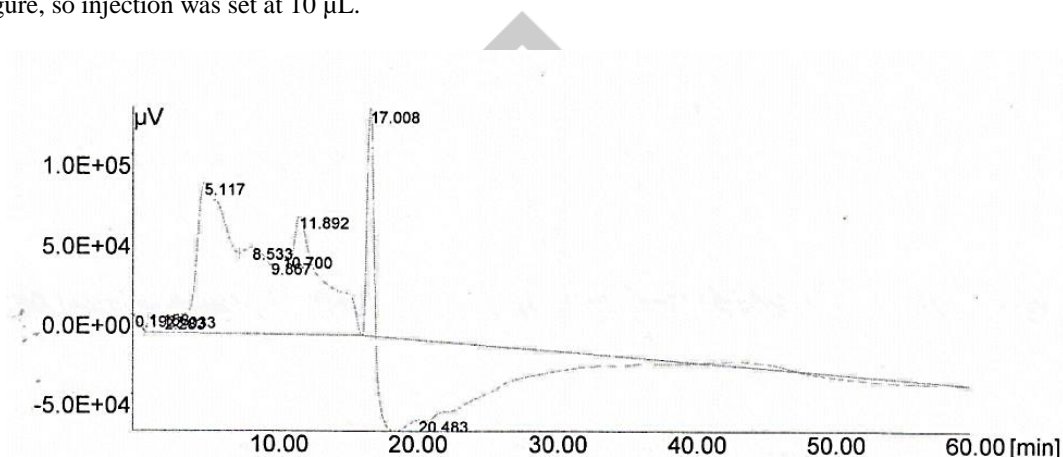
Fig 2. Mankozeb 80% 3 ppm standard solution chromatogram in acetonitrile :water 50:50 composition

According to table 1, the acetonitrile:water mixture (50:50) appeared in the shortest retention period and produced the greatest area. The 40:60 ratio produces a large enough area but with a longer retention duration. As a result, a 50:50 acetonitrile mixture was adopted. Table 2 shows the effect of a 50:50 acetonitrile mixture on retention time and area.

Table 2. Result of acetonitrile composition of 50:50 to retention time and area

Flow rate mobile phase (mL/minute)	Retention Time (minute)	Area (mAU*s)
0.30	2.027	847.47362
0.50	1.847	374.58362
0.70	0.963	103.49743
0.90	0.538	35.49583

The greater the solvents flow rate, the shorter the retention period and the lower the peak area of the chromatogram result. The best chromatogram with the shortest retention time was achieved at a flow rate of 0.30 mL/min, and the data revealed that the resultant chromatogram became asymmetrical at a flow rate greater than 0.70 mL/min. Furthermore, the peak area of the Mankozeb standard solution chromatogram was studied, and it was discovered that the greater the sample injection volume, the greater the chromatogram peak area, but at the injection volume 15 μ L, the chlorpyrifos standard solution chromatogram becomes asymmetrical, as shown in the following figure, so injection was set at 10 μ L.

Fig 3. Mankozeb 80% 3ppm chromatogram in injection volume of 15 μ L

Furthermore, precision was determined using three repetition in standard solutions based on the repeatability of the studied chromatogram area. If the relative standard deviation or coefficient of variation is less than 2%, the precision criteria is used. The regression equation derived from the calculations is: $Y = 0.000035 + 0.00167 X$, with a coefficient of variation of 1.12%. (3 ppm). HPLC measurements revealed a LOD value of 0.67 ppm and a LOQ value of 2.24 ppm.

3.2. Residual yield under optimal storage conditions

The application of ozonation at various ppm levels (table 3) produced results that were not substantially different from the 80 percent Mankozeb residue, but were considerably different from the control (without ozonation). Melon without ozonation had an 80 percent Mankozeb residual of 0.5875 mg/kg after three days of storage, whereas melon treated with ozone at 1.6 ppm (20 minutes contact time) had an 80 percent Mankozeb residue of 0.1682 mg/kg (3 days storage). Before the fourth day, all fruits had spoiled in room temperature storage, and there was an increase in pH but not significant.

Table 3. Mankozeb Residue Test Results at Various Treatment Levels

Contact and storage time	Ozone Concentration (ppm) on Mankozeb Residue		
	Results (mg/kg)		
	1.6	0.8	0.4
10 min. (3 days)	0.2844	0.5281	0.4031
20 min. (3 days)	0.1682	0.1992	0.5812

3.3. Microbial Analysis

Based on 719 sequences and after blast at NCBI, the results of the identification of 16S rRNA in melons under optimal conditions revealed interesting data, indicating that the *Kocuria rhizophila* (gram positive) strain was very dominant, presumably due to fungal resistance to pesticide active ingredients. Figure 4 depicts the electrophoresis findings in the form of the bacteria's molecular weight.

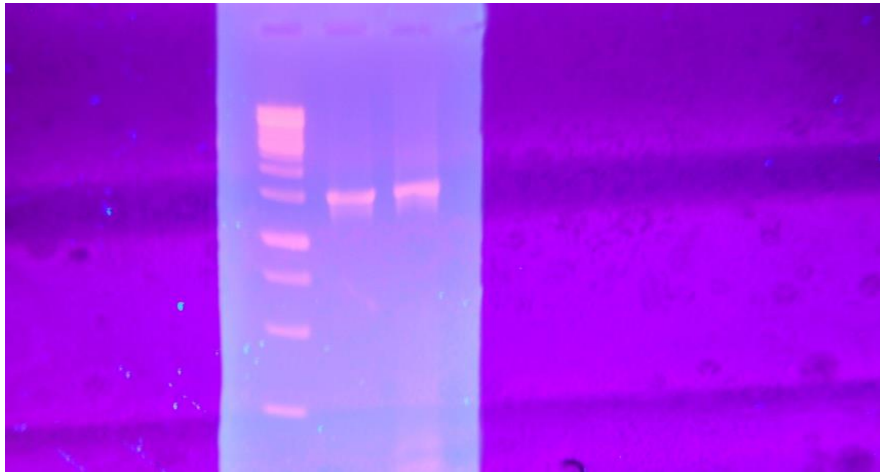


Fig 4. Electrophoresis result of *Kocuria rhizophila*

Primer 283F 5' – GAG AGT TTG ATC CTG GCT CAG GAC – 3' and Primer 261R 5' – AAA GGA GGT GAT CCA GCC GC – 3' were used to successfully read 719 16S rRNA sequences. The sequence of bases acquired is as follows, with the electropherogram reading results shown in Figure 5.

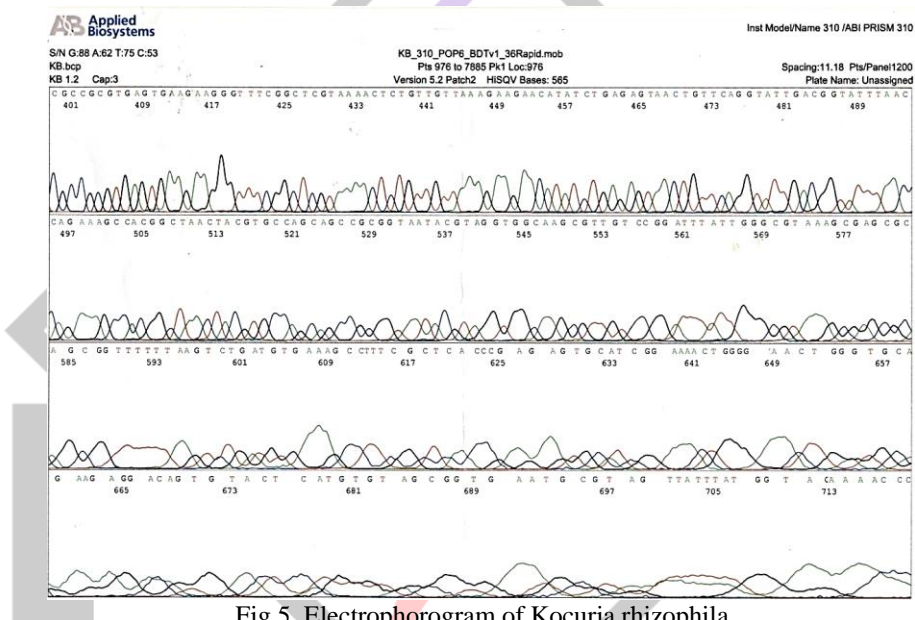


Fig 5. Electropherogram of *Kocuria rhizophila*

IV. CONCLUSION

The results indicated that ozonation at various ppm levels produced results that were not significantly different from the 80 percent Mankozeb residue, but were considerably different from the control (without ozonation). Melon without ozonation had an 80 percent Mankozeb residual of 0.5875 mg/kg after three days of storage, whereas melon treated with ozone at 1.6 ppm (20 minutes contact time) had an 80% Mankozeb residue of 0.1682 mg/kg (storage time). three days). The identification of bacteria in O1T2S1 treatment using 16S rRNA with 719 sequences and after blasting at NCBI revealed that the *Kocuria rhizophila* strain (gram positive) was prevalent, possibly owing to fungal resistance to pesticide active ingredients.

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