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Research Article

Hygienic Evaluation of Electrolyzed or Ozonated Water-Washed Apples Stored under Different Temperatures and Relative Humidity Conditions

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The presence of spoilage and pathogenic microorganisms is a concern for the safety of apples. In the present study, we evaluated the hygienic status of electrolyzed water (EW)- or ozonated water (OW)-washed apples, which were stored over 2 weeks under the combination of 2 different temperatures (25 and 30°C) and 2 different relative humidity (RH) conditions (85 and 90%). The average numbers of bacteria or fungi from unwashed and washed apples (EW or OW) did not show statistically significant differences at storage for 0, 1, or 2 weeks and had an increased tendency as the storage temperature, RH, and period increased. Identification of fungal isolates from apples revealed 3 main genera (*Fusarium* sp., *Trichoderma* sp., and *Alternaria* sp.) together with 8 minor genera (*Meyerozyma* sp., *Aspergillus* sp., *Glomerella* sp., *Neofusicoccum* sp., *Penicillium* sp., *Hypoxylon* sp., *Talaromyces* sp., and *Coprinellus* sp.). Moreover, sensory tests using EW- or OW-washed apples showed that OW did not significantly affect 5 quality characteristics (appearance, taste, flavor, texture, and overall acceptability). Our data suggest that EW or OW washing did not significantly reduce the levels of microorganisms on apples relative to the unwashed and that EW or OW washing did not deteriorate the quality of washed apples.

1. Introduction

The contamination of fresh produce such as fruits and vegetables with pathogenic microorganisms is a challenge to the safety of the food because it greatly increases the possibility of food-borne disease outbreaks. Typically, chlorine has been used as a sanitizing agent to reduce the number of microorganisms on fruits and vegetables [1, 2]. Approximately 50 to 200 mg/L of total chlorine concentration and a pH 6 to 7.5 in processing water were recommended to maintain a high level of hypochlorous acid [1, 3]. However, several studies reported that the efficacy of chlorine as a sanitizer at the recommended concentration for the reduction of initial microbial loads on fresh produce is very limited because it can generally reduce the population of spoilage microorganisms by only 1-2 log CFU/g [4, 5]. Other studies also showed that the accumulation of plant debris and exudates in washing water often leads to increased chlorine consumption, resulting in an increased potential for pathogen survival and cross-contamination, and that continuous replenishment of chlorine into the high organic washing water can generate carcinogenic halogenated compounds including trihalomethane [2, 6]. Recently, other washing water treatments such as ozonation and electrolysis were developed as an alternative sanitizing method to chlorination and are currently being used to produce ready-to-eat fruits and vegetables [1].

Ozone is a chemically active triatomic allotrope (O_3) of elemental oxygen, which can be generated by ultraviolet radiation and corona discharge [7]. After it was approved as a generally recognized as safe (GRAS) substance by the US Food and Drug Administration (FDA) in 2001, ozone has been commercially used as a disinfectant and sanitizer in food handling [8]. It has been applied in the aqueous or gaseous state to extend the shelf life of fresh produce due to its wide spectrum of antimicrobial properties that are

effective against bacteria, fungi, protozoa, and viruses as well as bacterial and fungal spores [9]. The antimicrobial effect of ozone is mainly due to the degradation of the bacterial cell membrane by the oxidation of thiol groups of cysteine residues in bacterial proteins and the oxidative process of polyunsaturated fatty acids to peroxides, which lead to leakage of cell contents and cell lysis [7, 8, 10]. Ozone has several advantages over other chemical sanitizers [10]. It spontaneously decomposes into nontoxic oxygen and leaves no residues on the surface of food. It also causes negligible loss of nutrients and sensory qualities in food [10].

Electrolyzed water (EW) is generated by electrolyzing a dilute sodium chloride (NaCl) solution with a current across an anode and cathode that are separated by a bipolar membrane. Electrolysis of the salt solution can produce strong biocidal substances such as hypochlorous acid (HOCl), hypochlorite ion (OCl^-), hydroxyl radical (·OH), and superoxide radical ($O_2\cdot^-$) [1]. After electrolysis, acidic electrolyzed water (AEW) containing 10–90 μg/mL of the chlorine concentration (pH 2-3) is produced at the anode, while alkaline electrolyzed water (ALEW; pH 10–13) is produced at the cathode [11]. Slightly acidic electrolyzed water (SAEW) containing 10–30 μg/mL of the chlorine concentration (pH 5-6) can be produced by electrolysis of NaCl or HCl in an electrolytic cell without a diaphragm. In the nondiaphragm electrolytic cell, slightly alkaline electrolyzed water or neutral electrolyzed water (NEW; pH 7-8) is also produced [11]. It has been reported that AEW and SAEW are widely used in disinfection and preservation of the fruits and vegetables [12]. In particular, SAEW contains about 95% HOCl (the most powerful antimicrobial chlorine form), 5% OCl^-, and trace amounts of Cl_2 as chlorine species [1]. Several studies have suggested the antimicrobial mechanism of EW. Zhang and collaborators described that OCl^- causes lysis of the external cell wall and cell membrane of microorganisms [11]. Other studies documented that oxidation of sulfhydryl groups in the presence of a high oxidation reduction potential (ORP) of AEW (>1100 mV) or SAEW (approximately 850 mV) can cause damage of the cell membrane and allow better permeability of HOCl through the membrane, resulting in cell death by destruction of nucleic acid and protein inside microorganisms [13, 14]. Also, HOCl and OH can induce oxidative decarboxylation of amino acids to nitrites and aldehydes, which disrupt protein synthesis [14]. Many researchers have reported the potential use of EW as a chlorine substitute for washing fresh fruits and vegetables [2, 13]. It is active against a broad spectrum of bacteria, fungi, and viruses and leaves less adverse chemical residues compared to chlorine [15].

A number of studies [1, 2, 9] have shown that OW and EW can effectively control a variety of pathogenic and spoilage microorganisms on fresh fruits and vegetables and that these treatment methods are currently used for disinfection of fresh produce in the food industry. In the fresh produce industry, it has been reported that the washing treatments with the water are usually applied in two different ways: dipping (or soaking) into the water and spraying (or rinsing) with the water [1, 2, 16]. Moreover, one study showed that rinsing of the fresh-cut vegetables with EW was more effective than dipping them in the same washing water [17]. Currently, OW-washed or EW-washed apples, which were sprayed with each washing water, are marketed as ready-to-eat fruits. In addition, the surrounding temperature and RH of the marketed apples are 2 main factors that affect the growth and survival of microorganisms on the surface of apples [18]. Thus, in this study, we evaluated the hygienic status of OW- or EW-spray-washed apples stored over 2 weeks (the average commercial storage period) at different temperatures (25 and 30°C) and relative humidity (RH) conditions (85 and 90%). In addition, we investigated the mycobiota of fungal pathogens, which can grow on and contaminate the surface of apples, and attempted to isolate patulin-producing fungi from the apples because apples are often contaminated with patulin, a mycotoxin.

2. Materials and Methods

2.1. Chemicals and Reagents. Tween 80, Proteinase K, ethanol, ethylenediaminetetraacetic acid (EDTA), tetracycline, and chloramphenicol were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). KCl, $BaCl_2$, KCl, KNO_3, and sodium acetate were obtained from Junsei Chemical Co. (Tokyo, Japan). Phenol/chloroform/isoamyl alcohol (25 : 24 : 1, PCI) were purchased from Biochemicals Inc. (Gyeonggi, South Korea). Tris base, 2-mercaptoethanol, and sodium dodecyl sulfate (SDS) were obtained from Bio rad (Hercules, CA, USA).

2.2. Plant Materials. Fresh fruits of one apple cultivar (Fuji), which were harvested, unwashed, or washed with EW or OW, and marketed in the spring of 2021, were used for evaluation of their microbiological hygienic status. The EW- or OW-washed apples and unwashed apples, which had a uniform size and weight without any damage or defects, were purchased from H-Grower Association (Cheongsong, Kyeongsangbuk, South Korea) and F-D Co. (Munkyeong, Kyeongsangbuk, South Korea), respectively.

2.3. Apple Storage Conditions. The apple samples wrapped with sterile plastic bags (3M PE sterile sample bag; St. Paul, MN, USA) were placed in covered containers (22.5 × 15.3 × 12.7; Interpark Holdings, Seoul, South Korea; 3 unwashed apples and 3 EW- or OW-washed apples in one container). The containers were adjusted to approximately 85 or 90% RH using saturated salt solutions (KCl for 84.5% RH and $BaCl_2$ for 90% RH at 25°C, KCl for 84.5% RH and KNO_3 for 91.5% RH at 30°C) [19, 20]. The containers were then stored at 25 or 30°C for 1 or 2 weeks (maximum storage period). We chose these RH and temperatures based on the data on climate conditions (average RH: 85%, average temperature: 30°C) during the summer in Seoul, South Korea.

2.4. Microbiological Analyses of Apple Surface. For microbiological analyses, the stem and blossom pits of each apple sample were rinsed with 200 μL of 0.85% sterile saline solution for each part (1.5 cm^2). For total aerobic plate counts

(APC), after half of the rinsed solution (100 μL) was 10-fold serially diluted with the sterile saline solution, 200 μL of the diluted solution was inoculated onto nutrient agar (NA; MB cell, Seoul, South Korea), and incubated at 37°C. The total number of aerobic bacteria was counted and calculated after a 2-day incubation. For total fungal counts, the rest of the rinsed solution (100 μl) was inoculated onto potato dextrose agar (PDA; MB Cell, Seoul, Korea) containing 2 types of antibiotic solutions (1 mg of tetracycline and 1 mg of chloramphenicol in 200 mL PDA) and incubated at 30°C. The total number of fungi was counted and calculated after 5 days of incubation [21].

2.5. Identification of Fungal Isolates and Phylogenetic Analysis. Fungal isolates, which were selected on PDA agar plates, were identified using DNA sequencing of the internal transcribed spacer 1 (ITS1)-5.8S rDNA-ITS2 region on fungal rDNA [22].

For genomic DNA isolation, approximately 10^8 of fungal spores were inoculated into 100 mL of potato dextrose broth (PDB; MB Cell, Seoul, Korea) in a 250 mL flask and incubated at 30°C for 4 days with shaking at 150 rpm after spores were prepared with a 0.01% Tween 80 solution from fungal isolates cultured on PDA agar plates. Mycelia were then lyophilized using a freeze-dryer (FD850; Ilshin Bio Branch, Seoul, South Korea) after they were filtered through miracloth (Sigma-Aldrich Co., St. Louis, MO, USA) on a Buchner funnel (Daihan Scientific, Wonju, Gangwon, South Korea). Genomic DNA isolation from fungal mycelia was performed by a procedure of Steven B Lee and John W. Taylor using phenol/chloroform/isoamyl alcohol with minor modification [23]. The ITS region of the isolated genomic DNA was amplified using 2 specific primers (ITS1 and 4) for the identification of fungal species. The primer sequences are as follows: ITS1 (5′-TCCGTAGGTGAACCTGCGG-3′, forward) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′, reverse). Polymerase chain reaction (PCR) was performed at 95°C for 5 min, followed by 35 cycles of 95°C for 1 min (denaturation), 55°C for 1 min (annealing), 72°C for 2 min (extension), and 72°C for 10 min (final extension). The PCR products were separated on 1.2% (w/v) agarose gels, purified using the AccuPep PCR/Gel Purification Kit (Bioneer, Daejeon, Korea), and sequenced by Biofact Co. (Daejeon, South Korea). Then, the fungal isolates were identified by the local similarity between DNA sequences of the PCR products and DNA sequences of fungal strains retrieved from GenBank at the National Center for Biotechnology Information (NCBI).

The phylogenetic tree was constructed using DNA sequences of identified fungal species and the MEGAX program based on the neighbor-joining (NJ) method [24].

2.6. Sensory Evaluation of EW- or OW-Washed Apples. The sensory evaluation of EW- or OW-washed apples was conducted by 10 untrained panelists (average age 23) for each washed apple group. The apple samples ($20 \times 20 \times 20$ mm) marked with a random 3-digit number were supplied to each panelist. The panelists evaluated the apple samples based on their appearance, flavor, taste, texture, and overall acceptability using a 10-point hedonic scale method (1 point = extremely bad, 5 points = fair, and 10 points = extremely good) [21].

2.7. Statistical Analyses. Data were statistically analyzed by *t*-test or a one-way analysis of variance (ANOVA) and expressed as the mean ± standard deviation using the SigmaStat software (Jandel Corporation, San Rafael, CA, USA). A p value <0.05 was considered statistically different.

3. Results and Discussion

3.1. Analyses of Total Aerobic Bacteria from Unwashed or EW-Washed Apples Stored under Different Temperatures and RH Conditions. We investigated the effects of temperature and RH on the level of total aerobic bacteria on unwashed or EW-washed apples during 2 weeks of storage under the combination of 2 different temperatures (25 and 30°C) and 2 different RH conditions (85 and 90%). The total APC was 1.06 ± 0.11 log CFU/cm^2 from unwashed apples without storage, while that was 1.04 ± 0.05 log CFU/cm^2 from EW-washed apples without storage (Figure 1(a)). There was no statistically significant difference ($p < 0.05$) between the total APC from unwashed and EW-washed apples without storage. In addition, when unwashed and EW-washed apples were stored at 25°C and 85% RH, 25°C and 90% RH, 30°C and 85% RH, or 30°C and 90% RH for 1 week, the total APC did not show statistically significant differences between the unwashed and EW-washed apples (Figure 1(b)). When apples were stored for 2 weeks under the same temperature and RH conditions as those for storage for 1 week, a comparison between the total APC from unwashed and EW-washed apples stored for 2 weeks showed a similar pattern to the results from apples stored for 1 week. When unwashed and EW-washed apples were stored at 25°C and 85% RH, 25°C and 90% RH, 30°C and 85% RH, or 30°C and 90% RH for 2 weeks, the total APC did not show statistically significant differences between the unwashed and EW-washed apples (Figure 1(c)). These results indicate that there were no effects of EW washing on bacteria that contaminated the surface of apples stored at the same temperature or under the same RH. In addition, for a given type of apple samples (unwashed or EW-washed) and RH condition (85 or 90%), the total APC was not significantly different after 1 week of storage at 25 and 30°C, while it had an increased tendency after 2 weeks of storage at 30°C compared to that at 25°C (Figures 1(b) and 1(c)). In particular, the total APC from the unwashed apples stored at 30°C and 90% RH for 2 weeks significantly increased, compared to that stored at 25°C and 90% RH conditions for 2 weeks ($p < 0.05$) (Figure 1(c)). It indicates that when apples were stored for 2 weeks, bacteria growth rates increased as the storage temperature increased to 30°C from 25°C under 90% RH, which promotes bacteria growth. Similarly, when apples stored at 30°C under 90% RH for 2 weeks were compared with those stored at 30°C under 85% RH for 2 weeks, the total APC from the apples stored at 30°C under 90% RH for 2 weeks had an increased trend relative to that from apples stored at 30°C under 85% RH

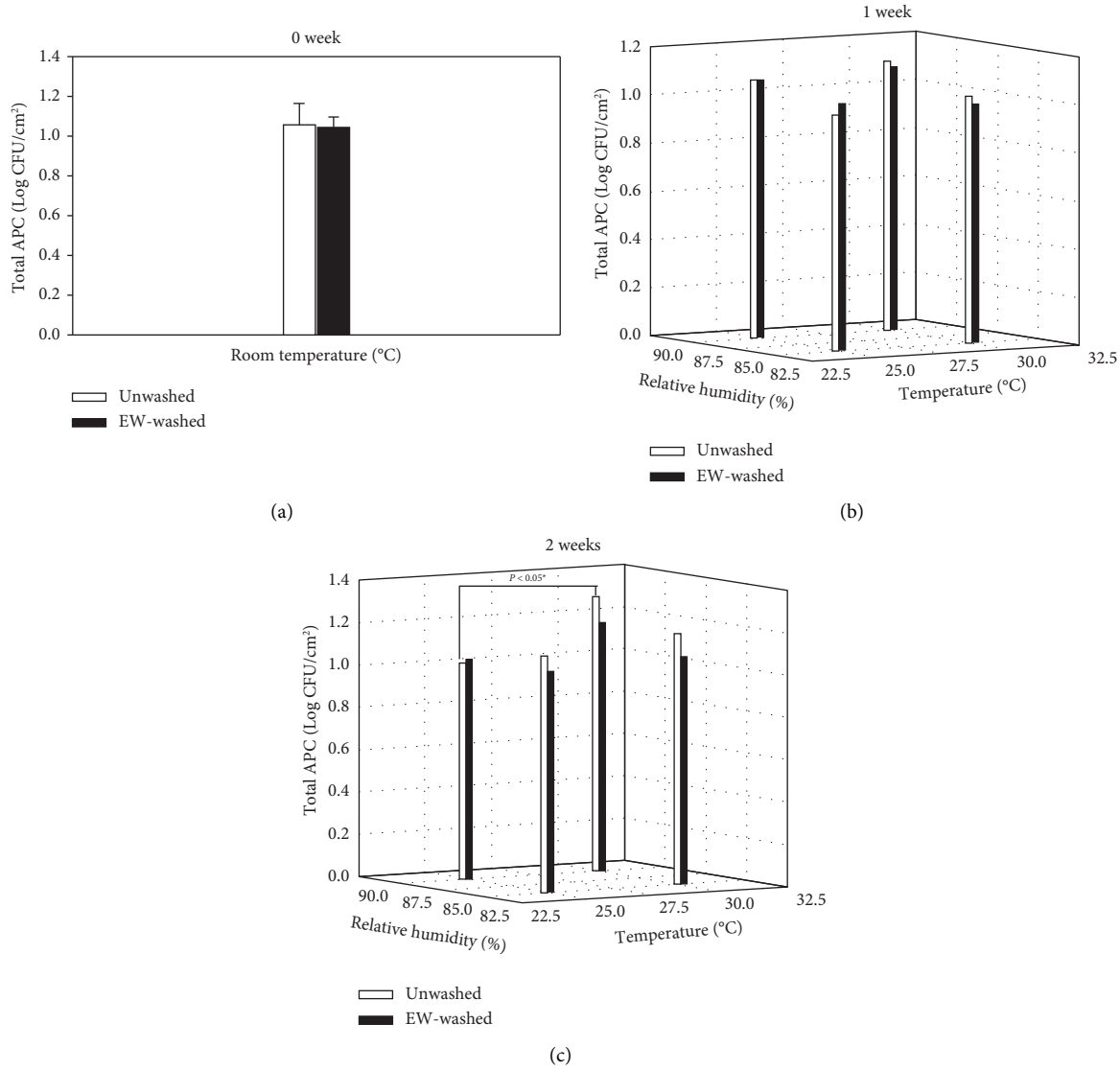


Figure 1: Levels of bacteria from unwashed or EW-washed apples stored for 1 week or 2 weeks under different temperature and RH conditions. (a) Unwashed and EW-washed apples at 0 week, (b) unwashed and EW-washed apples after 1 week of storage under the combination of 25 and 30°C and 85 and 90% RH, and (c) unwashed and EW-washed apples after 2 weeks of storage under the combination of 25 and 30°C and 85 and 90% RH. The levels of bacteria were measured in triplicate. The values are expressed as the mean ± standard deviation.

conditions for 2 weeks (Figure 1(c)). It suggests that when apples were stored at 30°C for 2 weeks, bacteria growth rates increased as the storage RH increased to 90% from 85%. Also, for a given type of apple sample (unwashed or EW-washed) and RH condition (85 or 90%), the total APC had an increased trend after 2 weeks of storage at 30°C, compared to that after 1 week of storage at the same temperature (Figures 1(b) and 1(c)). It indicates that bacteria growth rates increased as the length of storage at 30°C increased to 2 weeks from 1 week.

Two types of AEW have been approved by the Ministry of Food and Drug Safety in Korea for use as indirect food additives in the food industry: AEW and SAEW. SAEW is widely used for washing fresh fruits and vegetables [25]. In fruits and vegetables treated with SAEW, the initial bacterial population was reduced by 1-2 log CFU/g through 5–10 min exposure [26, 27]. One study documented that the total APC on the surface of apples was decreased by 1.89 log CFU/one fruit when those were treated with SAEW for 3 min [28]. In addition, it is known that the reduction of microbial contamination on the surface of food products was not as great as that obtained in suspension due to cell adherence onto the surface [13]. The main disadvantage of EW is that the sterilization and preservation effects are weak on fresh fruits when applied to them alone [11]. Several studies showed that a combination of EW with other postharvest treatments can effectively reduce contamination by spoilage microorganisms [29, 30]. A study described by Koseki and coworkers

showed that the combination of AEW, ALEW, and mild heat had a better bactericidal effect on lettuce than individual treatment [29]. Also, Hao and collaborators reported a reduction in the number of native microflora (total aerobic bacteria, coliforms, and yeasts and molds) on fresh-cut cilantro after sequential washing with ALEW followed by AEW for 5 min each [30]. In our study, the combination of AEW and SAEW instead of only SAEW treatment may have reduced the total APC. Moreover, Zhang and collaborators documented the reduction of 2 log CFU/apple for *Listeria monocytogenes* when fresh apples were dipped in SAEW for 3 min [11]. Thus, in our study, the exposure time (spraying for 1 min) of apples to SAEW may not have been enough to reduce levels of total APC on apples.

3.2. Analyses of Fungi from Unwashed or EW-Washed Apples Stored under Different Temperatures and RH Conditions. Since the growth and survival of food-borne fungi on the surface of apples are also affected by storage temperature and RH, we also investigated the effects of temperature and RH on the level of fungi on the unwashed or EW-washed apples during 2 weeks of storage under the combination of 2 different temperatures (25 and 30°C) and 2 different RH conditions (85 and 90%). The average number of fungi was 2.67 ± 0.67 CFU/cm^2 from unwashed apples without storage, while that was 3.56 ± 1.02 CFU/cm^2 from EW-washed apples without storage (Figure 2(a)). There was no statistically significant difference between the numbers of fungi in unwashed and EW-washed apples without storage. In addition, when the unwashed and EW-washed apples, which were stored at 25°C and either 85% or 90% RH for 1 week, were compared with each other, there was no significant difference between the average levels of fungi (Figure 2(b)). However, a comparison between the average levels of fungi from unwashed and EW-washed apples stored at 30°C and 90% RH for 1 week showed a statistically significant difference ($p < 0.05$) although those from unwashed and EW-washed apples stored at 30°C and 85% RH for 1 week did not show a statistically significant difference between them (Figure 2(b)). Also, when unwashed and EW-washed apples were stored at 25°C and 85% RH, 25°C and 90% RH, 30°C and 85% RH, or 30°C and 90% RH for 2 weeks, there was no significant difference between the average numbers of fungi (Figure 2(c)). In addition, for a given type of apple sample (unwashed or EW-washed) and RH condition (85 or 90%), the average level of fungi had an increased tendency after 1-week storage at 30°C, relative to that at 25°C (Figure 2(b)). It indicates that when apples were stored for 1 week, fungal growth rates increased as the storage temperature increased to 30°C from 25°C. However, for a given type of apple sample (unwashed or EW-washed) and temperature (25 or 30°C), the average number of fungi was not significantly different between 85 and 90% RH (Figures 2(b) and 2(c)). Also, for a given type of apple sample (unwashed or EW-washed) and RH condition (85 or 90%), the average level of fungi had an increased trend after 2-week storage at 25°C, compared to that after 1-week storage at the same temperature (Figures 2(b) and 2(c)). It indicates that fungal growth rates increased as the length of storage at 25°C increased to 2 weeks from 1 week.

The efficacy of the two types of AEW (AEW and SAEW) is mainly determined by the type and concentration of the electrolyte, the presence of organic matter, the temperature, pH, and ORP of the washing water, and the exposure time to washing water [12]. One study reported that AEW containing 20–30 μg/mL of active chlorine required 15 min of exposure to inactivate an initial count of 1,000 *Aspergillus* spores in suspension [31]. In particular, the antimicrobial activity of SAEW will be depleted over processing time due to the decomposition of HOCl if the system is not constantly supplied with HOCl by electrolysis. The accumulation of organic matter during sequential washing may also reduce the antimicrobial activity of EW as chlorine reacts with the organic compounds and the concentration of HOCl can be depleted. Thus, the concentration of chlorine in washing water should be monitored to ensure the antimicrobial efficacy of EW during its production and applications because chlorine loss occurs rapidly. Moreover, the exposure time to EW plays a key role in reducing the microbial count in fresh produce. Koide and coworkers reported a reduction of 1.5 log CFU/g for total aerobic bacteria and 1.3 log CFU/g for yeasts and molds when fresh-cut cabbage was dipped in SAEW for 10 min [27]. Therefore, in our study, when taken together with the total APC results described above, the exposure time (spraying for 1 min) of apples to SAEW (50–100 μg/mL of chlorine concentration) may not have been enough to reduce levels of total bacteria and fungi on apples.

3.3. Identification of Fungi Isolated from Unwashed or EW-Washed Apples Stored under Different Temperatures and RH Conditions. A total of 369 fungi were isolated from the stem and blossom pits of unwashed and EW-washed apples stored under the combination of 2 different temperatures (25 and 30°C) and 2 different RH conditions (85 and 90%). The fungal isolates were grouped by morphological characteristics such as the size and color of the colony on PDA agar plates. Then, 20 fungal isolates from 13 groups were selected for genetic identification based on sequences of ITS1-5.8S rDNA-ITS2 region on fungal rDNA. The ITS region was successfully amplified by PCR using genomic DNA from all 20 fungal isolates. BLAST-based analysis showed that the sequence similarity of the fungal isolates to the database in NCBI ranged from 85% to 100% (Table 1). The results exhibited that apples, which were unwashed or EW-washed, were contaminated with a variety of fungi. The 20 fungal isolates were categorized into 7 genera: *Trichoderma* sp. (2 species and 6 strains), *Meyerozyma* sp. (1 species and 4 strains), *Aspergillus* sp. (2 species and 3 strains), *Fusarium* sp. (2 species and 2 strains), *Penicillium* sp. (1 species and 2 strains), *Alternaria* sp. (2 species and 2 strains), and *Glomerella* sp. (1 species and 1 strain) (Table 1). Thus, when a total of 369 fungal isolates were assigned to the 7 genera, *Trichoderma* sp. was the most predominant genus (115 CFU and 31.17%) among the 7 genera, followed by *Fusarium* sp. (98 CFU and 26.56%), *Alternaria* sp. (58 CFU and 15.72%),

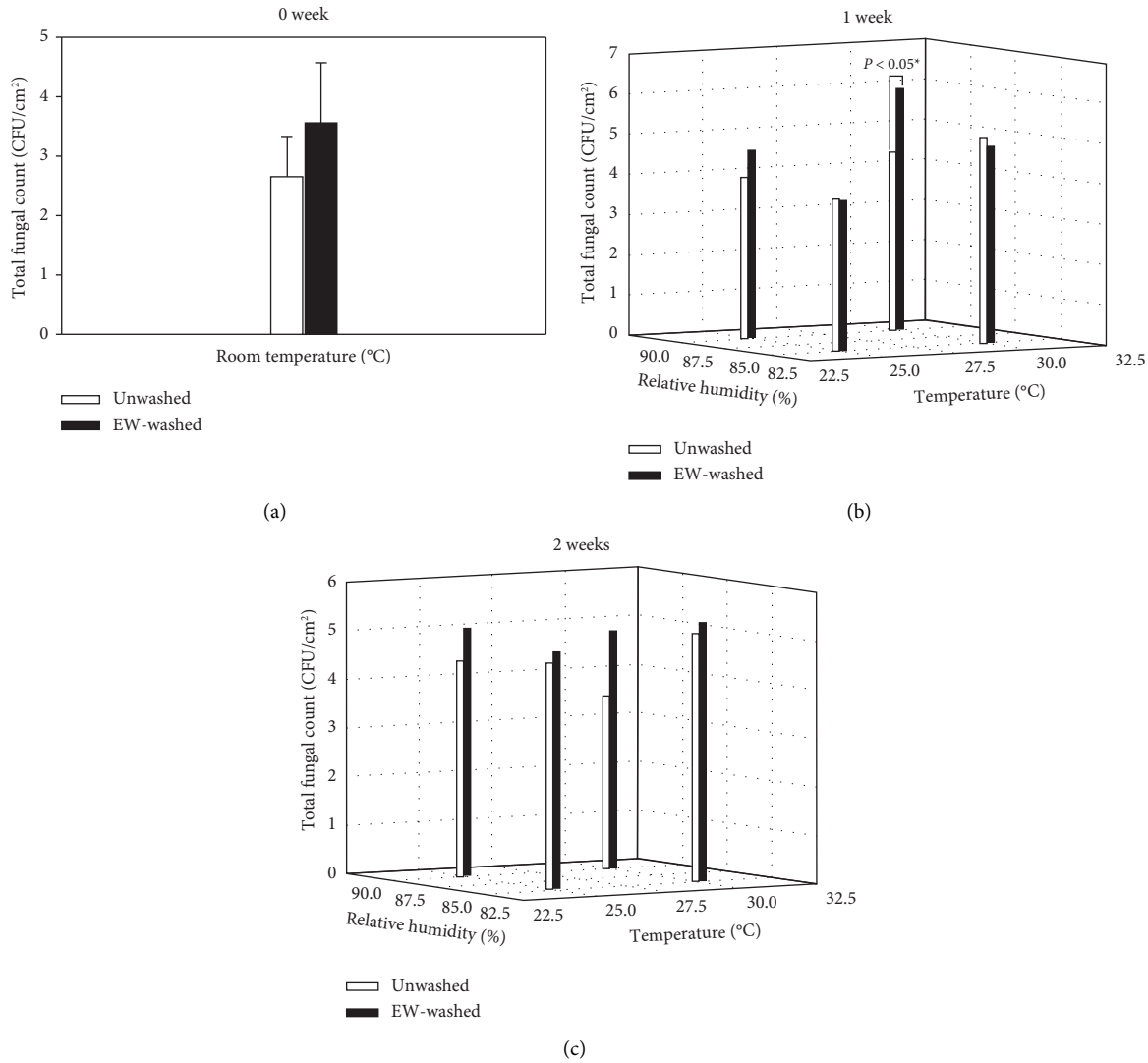


Figure 2: Levels of fungi from unwashed or EW-washed apples stored for 1 week or 2 weeks under different temperature and RH conditions. (a) Unwashed and EW-washed apples at 0 week, (b) unwashed and EW-washed apples after 1 week of storage under the combination of 25 and 30°C and 85 and 90% RH, and (c) unwashed and EW-washed apples after 2 weeks of storage under the combination of 25 and 30°C, and 85 and 90% RH. The levels of fungi were measured in triplicate. The values are expressed as the mean ± standard deviation.

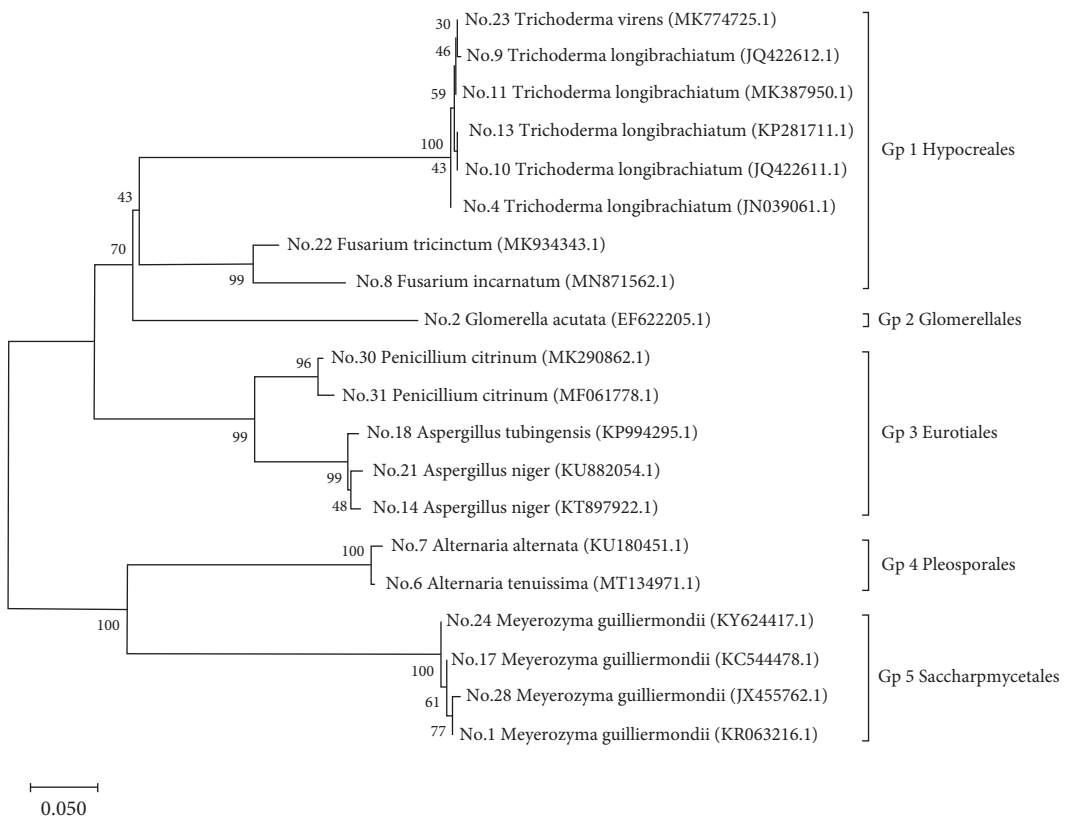
Meyerozyma sp. (50 CFU and 13.55%), *Aspergillus* sp. (23 CFU and 6.23%), *Glomerella* sp. (23 CFU and 6.23%), and *Penicillium* sp. (2 CFU and 0.54%). However, no patulin-producing fungi such as *Penicillium expansum* were isolated from unwashed or EW-washed apples. The phylogenetic tree based on ITS sequences from 20 fungal isolates is shown in Figure 3(a). The 20 fungal isolates belonged to 5 orders (Hypocreales, Glomerellales, Eurotiales, Pleosporales, and Saccharomycetales).

3.4. Analyses of Total Aerobic Bacteria from Unwashed or OW-Washed Apples Stored under Different Temperatures and RH Conditions. In a similar way to the case of EW-washed apples, we investigated the effects of temperature and RH on the level of total aerobic bacteria on unwashed and OW-washed apples during 2 weeks of storage under 2 different temperatures (25 and 30°C) and 2 different RH conditions (85 and 90%). The total APC was 1.10 ± 0.09 log CFU/cm^2 from unwashed apples without storage, while that was 1.07 ± 0.1 log CFU/cm^2 from OW-washed apples without storage (Figure 4(a)). There was no statistically significant difference between the total APC from unwashed and OW-washed apples without storage ($p < 0.05$). In addition, when unwashed and OW-washed apples were stored at 25°C and 85% RH, 25°C and 90% RH, 30°C and 85% RH, or 30°C and 90% RH for 1 week or 2 weeks, no significant difference was found between the total APC from unwashed and OW-washed apples (Figures 4(b) and 4(c)). When apples stored at 30°C were compared with those stored at 25°C, the total APC from a given type of apple sample (unwashed or OW-washed) had an increased tendency after 1 or 2 week storage at 30°C and 90% RH relative to that at 25°C and 90% RH (Figures 4(b) and 4(c)). It indicates that when apples were

Table 1: Identification of fungi isolated from unwashed or EW-washed apples using BLAST-based analysis.

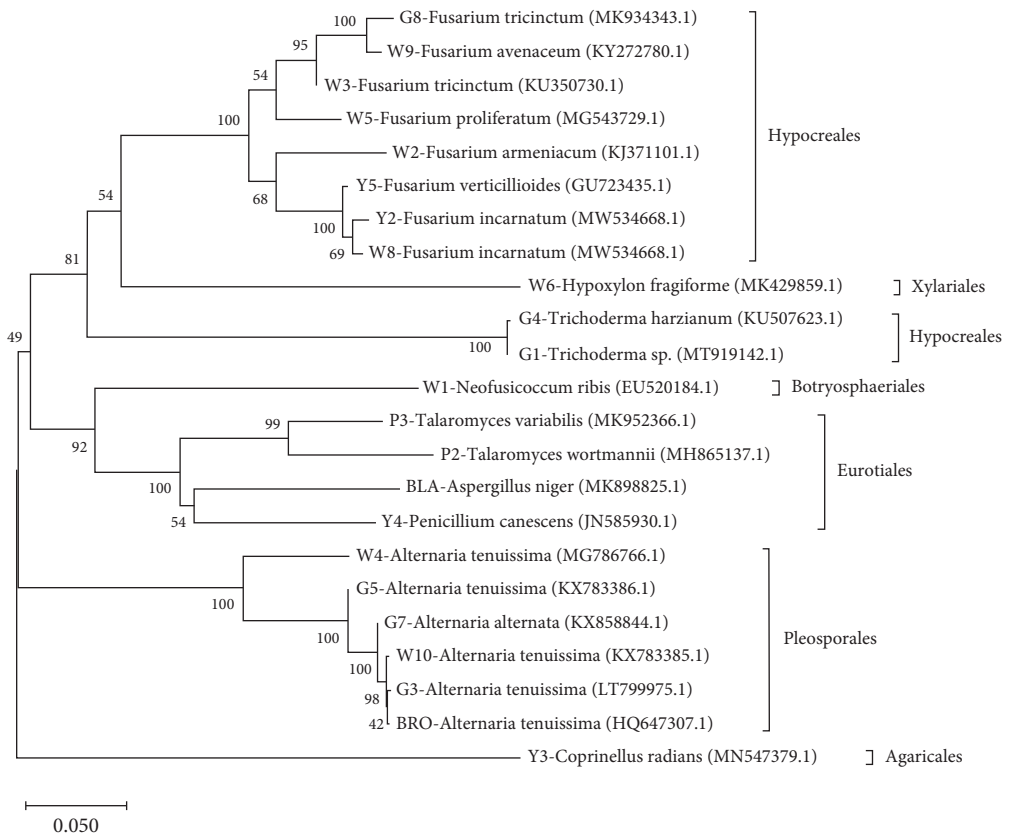
Sample IDs	Isolate or strain No. from NCBI	Scientific names (BLASTn accession no.)	Sequence similarity (%) (Length of aligned nucleotide sequence, bp)
No. 1	Strain n30a	*Meyerozyma guilliermondii* (KR063216.1)	99.0 (469/473 bp)
No. 17	Isolate B-WH X -12-09	*Meyerozyma guilliermondii* (KC544478.1)	99.0 (528/529 bp)
No. 24	Strain STR13	*Meyerozyma guilliermondii* (KY624417.1)	85.0 (159/186 bp)
No. 28	Strain EGV71	*Meyerozyma guilliermondii* (JX455762.1)	99.0 (463/468 bp)
No. 2	Strain UCA 116 0 NA	*Glomerella acutata* (EF622205.1)	99.0 (475/476 bp)
No. 4	Isolate TP4	*Trichoderma longibrachiatum* (JN039061.1)	100 (531/531 bp)
No. 9	Strain I-07	*Trichoderma longibrachiatum* (JQ422611.1)	99.0 (350/351 bp)
No. 10	Strain I-13	*Trichoderma longibrachiatum* (JQ422612.1)	99.0 (379/384 bp)
No. 11	Isolate 580855	*Trichoderma longibrachiatum* (MK387950.1)	98.0 (507/519 bp)
No. 13	Isolate F11	*Trichoderma longibrachiatum* (KP281711.1)	92.0 (345/375 bp)
No. 23	Isolate ET06_ ITS1	*Trichoderma virens* (MK774725.1)	88.0 (297/336 bp)
No. 6	Isolate N3L2	*Alternaria tenuissima* (MT134971.1)	100 (539/539 bp)
No. 7	Isolate K3	*Alternaria alternata* (KU180451.1)	99.0 (387/389 bp)
No. 8	Clone LS139	*Fusarium incarnatum* (MN871562.1)	100 (186/186 bp)
No. 22	Isolate FDW1	*Fusarium tricinctum* (MK934343.1)	100 (465/465 bp)
No. 14	Isolate MPb	*Aspergillus tubingensis* (KP994295.1)	99.0 (399/400 bp)
No. 18	Isolate AN-1	*Aspergillus niger* (KT897922.1)	99.0 (539/541 bp)
No. 21	Voucher USM SD2	*Aspergillus niger* (KU882054.1)	99.0 (350/352 bp)
No. 30	Strain ercha16	*Penicillium citrinum* (MK290862.1)	100 (505/505 bp)
No. 31	Strain SCAU116	*Penicillium citrinum* (MF061778.1)	99.0 (311/312 bp)

BLASTn was run using ITS1-5.8S rDNA-ITS2 sequences. BLASTn indicates basic local alignment search tool for nucleotide, whereas ITS represents internal transcribed spacer.



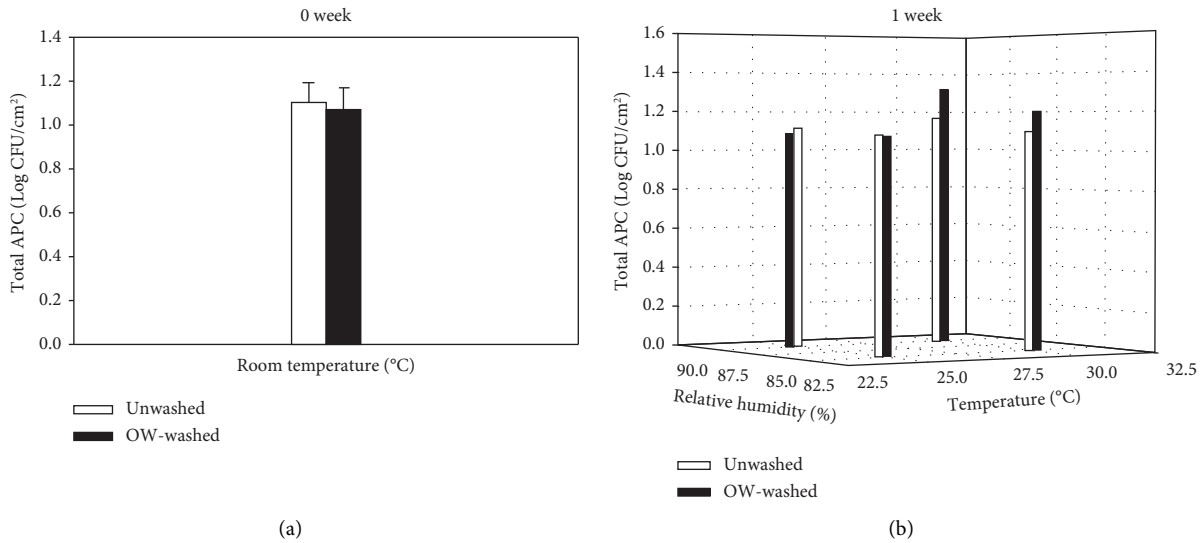
(a)

Figure 3: Continued.



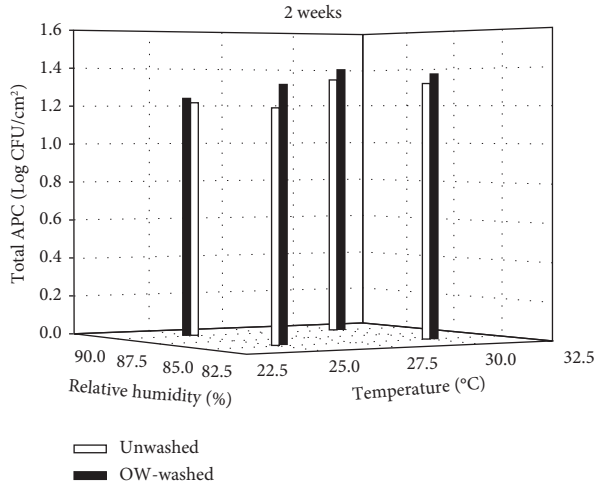
(b)

Figure 3: Phylogenetic relationship based on sequences of ITS1-5.8S rDNA-ITS2 region from fungi isolated from apples. (a) Phylogenetic tree of 20 fungi isolated from unwashed and EW-washed apples and (b) phylogenetic tree of 23 fungi isolated from unwashed and OW-washed apples. The trees were constructed using the neighbor-joining method.



(a) (b)

Figure 4: Continued.



(c)

Figure 4: Levels of bacteria from unwashed or OW-washed apples stored for 1 week or 2 weeks under different temperature and RH conditions. (a) Unwashed and OW-washed apples at 0 week, (b) unwashed and OW-washed apples after 1 week of storage under the combination of 25 and 30°C, and 85 and 90% RH, and (c) unwashed and OW-washed apples after 2 weeks of storage under the combination of 25 and 30°C and 85 and 90% RH. The levels of bacteria were measured in triplicate. The values are expressed as the mean ± standard deviation.

stored under 90% RH for 1 week or 2 weeks, bacteria growth rates increased as the storage temperature increased to 30°C from 25°C. Similarly, when apples stored at 30°C under 90% RH for 1 week were compared with those stored at 30°C under 85% RH for 1 week, the total APC from a given type of apple sample (unwashed or OW-washed) had an increased trend at 30°C under 90% RH, compared to that at the same temperature under 85% RH conditions (Figure 4(b)). It suggests that when apples were stored at 30°C for 1 week, bacteria growth rates increased as the storage RH increased to 90% from 85%. Also, for a given type of apple sample (unwashed or OW-washed), temperature (25 or 30°C), and RH condition (85 or 90%), the total APC after 2-week storage had an increased tendency, compared to that after 1-week storage (Figures 4(b) and 4(c)). It indicates that bacteria growth rates increased as the storage period increased to 2 weeks from 1 week under the combination of 2 different temperatures (25 or 30°C) and 2 different RH conditions (85 or 90%).

3.5. Analyses of Fungi from Unwashed or OW-Washed Apples Stored under Different Temperatures and RH Conditions. We also investigated the effects of temperature and RH on the level of fungi on the unwashed or OW-washed apples during 2 weeks of storage under the combination of 2 different temperatures (25 and 30°C) and 2 different RH conditions (85 and 90%). There was no statistically significant difference ($p < 0.05$) between the numbers of fungi from unwashed and OW-washed apples without storage (Figure 5(a)). In addition, when the unwashed and OW-washed apples, which were stored under the combination of 2 different temperatures (25 or 30°C) and 2 different RH conditions (85 or 90% RH) for 1 week or 2 weeks, were compared with each other, there was no significant difference ($p < 0.05$) between the average numbers of fungi (Figures 5(b) and 5(c)). However, when unwashed apples stored at 30°C for 1 week were compared with those stored at 25°C for 1 week, the average level of fungi increased after storage at 30°C under 85 or 90% RH relative to that after storage at 25°C under the same RH condition ($p < 0.05$) (Figure 5(b)). Similarly, in the case of OW-washed apples, when apples stored at 30°C were compared with those stored at 25°C, the average number of fungi had an increased trend after 1-or 2-week storage at 30°C under 85 or 90% RH relative to that at 25°C under the same RH condition (Figures 5(b) and 5(c)). These data indicate that when apples were stored for 1 week or 2 weeks, fungal growth rates increased as the storage temperature increased to 30°C from 25°C. However, for a given type of apple sample (unwashed or OW-washed) and temperature (25 or 30°C), the average level of fungi did not show any statistically significant differences after storage under 90% RH, compared to that after storage under 85% RH conditions (Figures 5(b) and 5(c)).

In general, the RH of the storage environment of food may affect its quality because it can lead to a change in its water activity. Eventually, food will come into moisture equilibrium with its surroundings. The water activity, equilibrated with the surrounding RH, on the surface of food will allow microbial growth, leading to food spoilage. In contrast to the fungal results, as described above, the total APC from unwashed or washed apples (treated with EW or OW) had an increased trend after 2-week storage at 30°C under 90% RH, compared to that at 30°C under 85% RH conditions (Figures 1(c) and 4(c)). It seems that fungal growth rates under 85 and 90% RH are not much different because the extreme water activity for fungal species is far lower (0.61) than that for bacteria (0.71) [32].

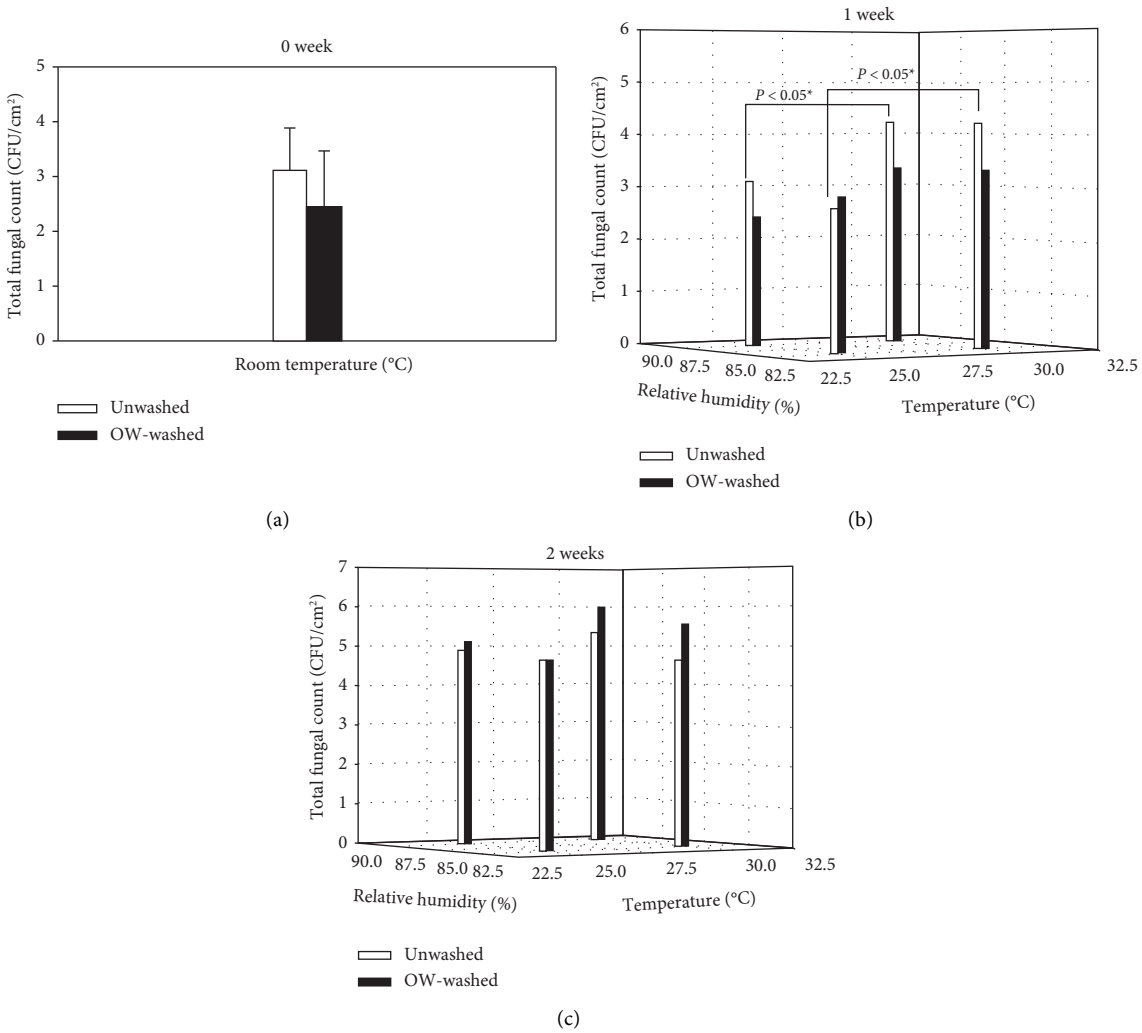


FIGURE 5: Levels of fungi from unwashed or OW-washed apples stored for 1 week or 2 weeks under different temperature and RH conditions. (a) Unwashed and OW-washed apples at 0 week, (b) unwashed and OW-washed apples after 1 week of storage under the combination of 25 and 30°C and 85 and 90% RH, and (c) unwashed and OW-washed apples after 2 weeks of storage under the combination of 25 and 30°C and 85 and 90% RH. The levels of fungi were measured in triplicate. The values are expressed as the mean ± standard deviation.

For a given type of apple sample (unwashed or OW-washed) and RH condition (85 or 90%), statistically significant differences were found between the average numbers of fungi after storage for 1 week and 2 weeks at 25°C ($p < 0.05$) (Figures 5(b) and 5(c)). Similarly, the comparison between the average levels of fungi from OW-washed apples after storage of 1 week and 2 weeks at 30°C also showed a statistically significant difference ($p < 0.05$) (Figures 5(b) and 5(c)). It indicates that fungal growth rates increased as the storage period increased to 2 weeks from 1 week.

Interestingly, there was a trend in which washed apples (treated with OW or EW) after 2 weeks of storage had a higher number of bacteria or fungi than unwashed apples (Figures 1(b) and 1(c), Figures 2(b) and 2(c), Figures 4(b) and 4(c), and Figures 5(b) and 5(c)). It is consistent with a previous study in which AEW treatment reduced the initial microbial population on fresh-cut vegetables and subsequent bacterial growth rates on them were higher than those on untreated vegetables [33]. The reason for this is likely that damaged spores or cells take more recovery time than 1 week, and the decreased initial microbial population provides plenty of room for microbial growth on EW- or OW-washed apples.

In general, ozone has some limitations such as rapid decomposition and reaction with food constituents, resulting in decreased amounts of residual ozone in washing water and ineffectiveness as an antimicrobial sanitizer [34]. The susceptibility of microorganisms to ozone varies with their physiological state, pH and temperature of ozonated washing water, exposure time treated with the water, and the RH of the facility [35]. Liu and coworkers showed that aqueous ozone treatments (1.4 mg/L) for 5 or 10 min reduced the number of total bacteria on fresh-cut apples by 1.83 and 2.13 log CFU/g compared to the control samples on the 12th day of cold storage [21]. Previous studies documented that aqueous ozone treatment for bacteria required

5–10 min exposure and that for fungi it needed longer exposure time [35, 36]. Naitoh and Shiga reported that the thresholds of antimicrobial activity of aqueous ozone (0.3–0.5 mg/L) against spores of *Aspergillus* sp., *Penicillium* sp., and *Candida* sp. were 90 to 180 min, 45 to 60 min, and 5 to 10 min exposure, respectively [36], which is consistent with one study that described that fungal spores appear quite resistant to ozone, compared to bacteria [37, 38]. Thus, in the present study, when taken together with the total APC results described above, it is likely that 0.47 mg/L of aqueous ozone sprayed onto apples for 0.5 min may not have been enough to reduce the numbers of total aerobic bacteria and fungi on apples. A longer exposure time to aqueous ozone would enhance the shelf life and microbiological safety of apples.

3.6. Identification of Fungi Isolated from Unwashed or OW-Washed Apples Stored under Different Temperatures and RH Conditions. A total of 326 fungi were isolated from the stem and blossom pits of unwashed and OW-washed apples stored under the combination of 2 different temperatures (25 and 30°C) and 2 different RH conditions (85 and 90%). Based on the size and color of the colony on PDA agar plates, the fungal isolates were grouped. Then, 23 fungal isolates were selected from 16 groups to identify their taxonomic names by sequencing the ITS1-5.8S rDNA-ITS2 region on rDNA of the fungal genome. After PCR amplification using genomic DNA from all 23 fungal isolates, BLAST-based analysis showed that unwashed and OW-washed apples were contaminated with various fungi along with 95%–100% sequence similarities between the DNA sequences of the fungal isolates and fungal strains retrieved from GenBank in NCBI (Table 2). The 23 fungal isolates were categorized into 9 genera: *Fusarium* sp. (6 species and 7 strains), *Alternaria* sp. (2 species and 6 strains), *Trichoderma* sp. (2 species and 2 strains), *Talaromyces* sp. (2 species and 2 strains), *Hypoxylon* sp. (1 species and 1 strain), *Neofusicoccum* sp. (1 species and 1 strain), *Aspergillus* sp. (1 species and 1 strain), and *Penicillium* sp. (1 species and 1 strain) (Table 2). Accordingly, a total of 326 fungal isolates were assigned to the 9 genera. *Fusarium* sp. (134 CFU and 41.10%) was the most frequent genus among the 9 genera, followed by *Alternaria* sp. (99 CFU and 30.34%), *Trichoderma* sp. (40 CFU and 12.27%), *Aspergillus* sp. (18 CFU and 5.52%), *Neofusicoccum* sp. (16 CFU and 4.91%), *Penicillium* sp. (13 CFU and 3.99%), *Hypoxylon* sp. (3 CFU and 0.92%), *Talaromyces* sp. (2 CFU and 0.61%), and *Coprinellus* sp. (1 CFU and 0.31%). These results are slightly different but mostly similar to the fungal data from unwashed or EW-washed apples, which were described above. In both cases, *Fusarium* sp., *Alternaria* sp., and *Trichoderma* sp. were the most prevalent genera. These results are consistent with those from other researchers [6, 39, 40].Tournas and coworkers reported that they isolated Alternaria sp., Cladosporium sp., Penicillium sp., and Fusarium sp. from apples (Fuji) collected from Maryland, USA [39]. Another study from Denmark showed that authors isolated some fungi such as *Alternaria tenuissima*, *Alternaria arborescens*, *Fusarium avenaceum*, *Fusarium lateritium*, *Penicillium crustosum*, and *Penicillium expansum* from moldy apples [6]. In addition, a study from Portugal showed that *Penicillium* sp., *Cladosporium* sp., *Alternaria* sp., *Fusarium* sp., and *Aspergillus* sp. were identified from rotten apples [40]. However, our data are different from one study from Saudi Arabia [41]. In their study, they identified 4 fungi (*Penicillium chrysogenum*, *Penicillium adametzii*, *Aspergillus oryzae*, and *Penicillium stekii*) from apples (Red Delicious or Granny Smith) stored at 25–30°C after harvested in Riyadh, Saudi Arabia. This discrepancy may have been attributed to differences in the geographical location, apple varieties, or climate. Abdelfattah and collaborators showed in the microbiome study of apples harvested from 8 countries that the geographical location (and orchards within a location) in which apples were harvested had a significant effect on the fungal diversity associated with the fruit [42]. One of the reasons for this discrepancy between fungi from EW-and OW-washed apples may be the fact that the apples were harvested from different orchards in different regions (Munkyeong for EW-washed apples and Cheongsong for OW-washed apples), the soil of which may not have contained the same fungal species although apples from both regions belonged to the same cultivar (Fuji). Another possibility is that EW and OW as sanitizers act differently on different fungal species. However, this is not likely the case because levels of microorganisms did not show significant differences between unwashed and washed apples (electrolyzed or ozonated). In addition, again unfortunately, no patulin-producing fungi such as *P. expansum* were isolated from unwashed and OW-washed apples. The phylogenetic tree based on ITS sequences from 23 fungal isolates is shown in Figure 3(b). The 23 fungal isolates belonged to 6 orders (Hypocreales, Xylariales, Botryosphaeriales, Eurotiales, Pleosporales, and Agaricales). These taxonomic orders to which fungi isolated from unwashed and OW-washed apples belong are slightly different from those to which fungi isolated in unwashed and EW-washed apples belong. Fungi which are classified into Glomerellales and Saccharomycetales were found only from unwashed and EW-washed apples, while those which are classified into Xylariales, Botryosphaeriales, and Agaricales were found only from unwashed and OW-washed apples. When taken together, most of the fungi, which were categorized into seven of the 8 orders from unwashed or EW- or OW-washed apples, belonged to 1 phylum (Ascomycota), while those in the other order (Agaricales) belonged to another phylum Basidiomycota. To the best of our knowledge, this is the first report on *Coprinellius radians*, which belongs to Basidiomycota, isolated from apples.

We reviewed 22 identified fungal species in the literature for their ability to produce mycotoxin production. Table 3 shows the summary of major mycotoxins that can be produced by the fungal species identified in this study. Most of them are mycotoxins from *Fusarium* sp., and some of them are from *Trichoderma* sp., *Alternaria* sp., *Aspergillus* sp., and *Penicillium* sp. One of our aims in this study was to isolate patulin-producing fungi from apples. Moslem and collaborators showed that *Penicillium canescens*, one of 22 fungal species identified in this study, may produce patulin as well

Table 2: Identification of fungi isolated from unwashed or OW-washed apples using BLAST-based analysis.

Sample IDs	Isolate or strain No. from NCBI	Scientific names (BLASTn accession no.)	Sequence similarity (%) (Length of aligned nucleotide sequence, bp)
BLA	Isolate 2-1	*Aspergillus niger* (MK898825.1)	100 (538/538 bp)
BRO	Strain HC-2	*Alternaria alternata* (MT644140.1)	100 (534/534 bp)
G7	Isolate aa001	*Alternaria alternata* (KX858844.1)	100 (471/471 bp)
G3	Isolate SA-PS	*Alternaria tenuissima* (LT799975.1)	100 (471/471 bp)
G5	Isolate ZB11263564	*Alternaria tenuissima* (LT799975.1)	99.8 (431/432 bp)
W4	Isolate NIR12	*Alternaria tenuissima* (MG786766.1)	99.0 (324/326 bp)
W10	Isolate ZB11060981	*Alternaria tenuissima* (KX783385.1)	99.8 (536/537 bp)
G1	Isolate ER 12	*Trichoderma* sp. (MT919142.1)	99.8 (537/538 bp)
G4	Strain MMCC 1581.2	*Trichoderma harzianum* (KU507623.1)	100 (524/524 bp)
G8	Isolate FDW1	*Fusarium tricinctum* (MK934343.1)	99.4 (520/523 bp)
W3	Strain WBS020	*Fusarium tricinctum* (KU350730.1)	100 (317/317 bp)
W8	Isolate J15_19	*Fusarium incarnatum* (MW534668.1)	100 (510/510 bp)
Y2	Isolate J15_19	*Fusarium incarnatum* (MW534668.1)	99.6 (513/516 bp)
W2	Isolate NH4982	*Fusarium armeniacum* (KJ371101.1)	98.0 (375/384 bp)
W5	Isolate LrBF11	*Fusarium proliferatum* (MG543729.1)	99.7 (372/373 bp)
W9	Isolate 29a	*Fusarium avenaceum* (KY272780.1)	99.8 (527/528 bp)
Y5	Isolate P6-26	*Fusarium verticillioides* (GU723435.1)	99.7 (389/390 bp)
W1	Isolate NW316	*Neofusicoccum ribis* (EU520184.1)	95.0 (408/431 bp)
W6	Isolate RY-3	*Hypoxylon fragiforme* (MK429859.1)	100 (526/526 bp)
Y3	Isolate 10	*Coprinellus radians* (MN547379.1)	98.0 (336/348 bp)
Y4	Strain Cs/1/2	*Penicillium canescens* (JN585930.1)	100 (528/528 bp)
P2	Strain CBS128881	*Talaromyces wortmannii* (MH865137.1)	96.0 (350/365 bp)
P3	Strain TvH5501	*Talaromyces variabilis* (MK952366.1)	98.0 (501/510 bp)

BLASTn was run using ITS1-5.8S rDNA-ITS2 sequences. BLASTn indicates basic local alignment search tool for nucleotide, whereas ITS represents internal transcribed spacer.

Table 3: Fungal species isolated in this study and major mycotoxins and toxicities that can be produced by them.

Fungal species	Mycotoxin	Toxicity	Reference
Trichoderma virens	Gliotoxin	Immunosuppression	[43]
Trichoderma longibrachiatum	Trilongins	Voltage-dependent ion channel damage	[44]
Fusarium tricinctum	T-2 toxin, zearalenone	Immunotoxicity, reproductive toxicity	[45, 46]
Fusarium incarnatum	Fumonisin, zearalenone	Neurotoxicity, reproductive toxicity	[47, 48]
Fusarium avenaceum	Zearalenone, moniliformin	Reproductive toxicity, immunosuppression, cytotoxicity	[48]
Fusarium proliferatum	Fumonisin, moniliformin	Neurotoxicity, immunosuppression, cytotoxicity	[48]
Fusarium armeniacum	T-2 toxin, HT-2 toxin	Immunotoxicity immunotoxicity	[49]
Fusarium verticilioides	Fumonisin, moniliformin	Neurotoxicity, immunosuppression, cytotoxicity	[50]
Alternaria tenuissima	Alternariol, tenuazonic acid	Immunotoxicity, immunotoxicity	[45]
Alternaria alteranata	Alternariol, tenuazonic acid	Immunotoxicity, immunotoxicity	[45]
Aspergillus niger	Ochratoxin	Nephrotoxicity	[58]
Aspergillus tubingensis	Ochratoxin	Nephrotoxicity	[59]
Penicillium citrinum	Citrinin	Nephrotoxicity	[60]
Penicillium canescens	Citrinin, patulin	Nephrotoxicity, genotoxicity	[51]
Talaromyces wortmannii	Rugulovasine	Cardiovascular toxicity	[61]

as citrinin [51]. Thus, we analyzed the culture extracts of *Penicillium canescens* by high performance liquid chromatography-UV detector (HPLC-UVD) to test for patulin production. However, we did not detect a patulin peak from the culture extract (data not shown). Our data showed that any patulin-producing fungi such as *P. expansum* were not isolated from apples (Fuji). This result may have been attributed to the fact that Fuji apples are relatively more resistant to patulin production due to their texture and weak acidity than other apple cultivars [52].

3.7. Sensory Evaluation of EW- or OW-Washed Apples. A sensory test was conducted to evaluate the appearance, taste, flavor, texture, and overall acceptability of unwashed or EW-washed apples. The scores for appearance, flavor, and overall acceptability of EW-washed apples were higher than those of unwashed apples, which were statistically significant ($p > 0.05$) (Table 4). These results are slightly different from previous studies [17, 53]. Izumi showed that SAEW (20 mg/L) did not significantly affect the quality of fresh-cut vegetables, such as the color and general

Table 4: Sensory evaluation of unwashed or EW-washed apples using a 10-point hedonic scale method.

Samples	Appearance	Taste	Flavor	Texture	Overall acceptability
Unwashed apples	5.80 ± 2.97^{a}	8.7 ± 0.82	7.90 ± 0.74^{a}	8.20 ± 1.23	6.70 ± 1.60
EW-washed apples	8.90 ± 0.99^{b}	8.10 ± 0.74	8.70 ± 0.82^{b}	8.60 ± 0.84	8.80 ± 0.79

Different letters in the same column indicate statistically significance between data ($p < 0.05$ analyzed by t-test). Data are expressed as the mean ± standard deviation, which were measured in triplicate (1 point = extremely bad, 5 points = fair, and 10 points = extremely good).

Table 5: Sensory evaluation of unwashed or OW-washed apples using a 10-point hedonic scale method.

Samples	Appearance	Taste	Flavor	Texture	Overall acceptability
Unwashed apples	4.60 ± 0.52	4.60 ± 0.52	4.70 ± 0.48	4.30 ± 0.48	4.60 ± 0.52
OW-washed apples	4.60 ± 0.52	4.50 ± 0.53	4.70 ± 0.48	4.70 ± 0.48	4.50 ± 0.53

Different letters in the same column indicate statistically significance between data ($p < 0.05$ analyzed by t-test). Data are expressed as the mean ± standard deviation, which were measured in triplicate (1 point = extremely bad, 5 points = fair, and 10 points = extremely good).

appearance of carrots and spinach [17]. Nimitkeatkai and Kim also reported that there was no significant difference in the sensory quality of apples treated with SAEW for 5 min [53]. Moreover, SAEW treatment was able to maintain the content of bioactive compounds such as total phenolics and flavonoids in peaches and blueberries [10, 54]. In addition, in the present study, of the 5 sensory characteristics, the highest score (8.90) and the second highest score (8.80) were obtained for the appearance and overall acceptability of EW-washed apples, respectively (Table 4). One of the possible reasons for the higher score in appearance for EW-washed apples is likely that the color of EW-washed apples was brighter than that of unwashed apples and that the stem and blossom pits of the former had less dirt than those of the latter. The visual quality of apples such as color is one of the important parameters in sensory evaluation because their good appearance is preferred by consumers [37]. Thus, the panelists may have preferred the appearance of EW-washed apples to other characteristics in the sensory evaluation, which in turn affected the overall acceptability of EW-washed apples.

Another sensory test was performed to evaluate the 5 characteristics of unwashed or OW-washed apples. There was no statistically significant difference between unwashed and OW-washed apples in their appearance, taste, flavor, texture, and overall acceptability (Table 5). It seems like OW did not have any effect on the 5 characteristics in the sensory evaluation. This is in agreement with previous studies in which they reported that ozone did not significantly affect the sensory qualities of fresh produce. Skog and collaborators described that apples treated with 0.4 mL/L of ozone in cold storage did not show any changes in texture, color, and taste [55]. Other studies also showed that there were no significant differences between unwashed and OW-treated apples in the overall sensory quality although some researchers reported a negative effect of ozone treatment on the quality of fruits and vegetables, such as the altered surface color of carrot [21, 56, 57]. In addition, considering the scores for 5 characteristics of the unwashed apples, the scores for unwashed apples as controls for OW and EW washing were different (Tables 4 and 5). The discrepancy could be due to different harvest regions for the unwashed apples.

Overall, EW-washed apples received higher scores than unwashed apples, while OW-washed apples received similar scores to those of unwashed apples. Therefore, when taken together with the microbiological analysis data described above, the EW-washing method is likely to be better than the OW-washing method.

4. Conclusions

Fruits and vegetables including apples are highly nutritious and provide health benefits to human, such as richness in fiber and antioxidants. The postharvest microbiological safety of apples plays an important role in human health. In this study, EW or OW washing did not have significant effects on the reduction of the levels of microorganisms on apples relative to unwashing and EW or OW washing did not deteriorate the quality of washed apples. Furthermore, our study showed that identification of fungal isolates from apples revealed 3 main genera (*Fusarium* sp., *Trichoderma* sp., and *Alternaria* sp.) together with 8 minor genera (*Meyerozyma* sp., *Aspergillus* sp., *Glomerella* sp., *Neofusicoccum* sp., *Penicillium* sp., *Hypoxylon* sp., *Talaromyces* sp., and *Coprinellus* sp.). Therefore, the concentration of chlorine in EW or ozone in OW in washing water and exposure time to them should be optimized to ensure their antimicrobial efficacy (longer than 1 min for SAEW (50–100 μg/mL of chlorine concentration) and longer than 0.5 min for OW [0.47 mg/L of ozone concentration)). In addition, maintenance of the decreased microbial load on the produce during storage is also important because the remaining microorganisms could grow rapidly after washing. Thus, the beneficial effects of EW or OW treatment should not be overestimated, and fruits and vegetables treated with EW or OW should be maintained at low temperature such as below 5°C. Accordingly, a more advanced and dynamic SAEW or OW production and application system that is capable of overcoming all the current limitations should be developed for the microbiological safety of EW- or OW-washed apples in the future. These may

include procedures for the application of on-farm food safety programs such as Good Agricultural Practices (GAP) and an in-plant food safety program such as Hazard Analysis and Critical Control Point (HACCP) and Sanitation Standard Operating Procedure (SSOP) systems.

Data Availability

The data supporting the current study are available from corresponding author upon request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

Jiao Wang and Yuequi Pei contributed equally to this work.

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