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Effectiveness of Commercial and Homemade Washing Agents in Removing Pesticide Residues on and in Apples

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Supporting Information

ABSTRACT: Removal of pesticide residues from fresh produce is important to reduce pesticide exposure to humans. This study investigated the effectiveness of commercial and homemade washing agents in the removal of surface and internalized pesticide residues from apples. Surface-enhanced Raman scattering (SERS) mapping and liquid chromatography tandem mass spectrometry (LC−MS/MS) methods were used to determine the effectiveness of different washing agents in removing pesticide residues. Surface pesticide residues were most effectively removed by sodium bicarbonate (baking soda, $NaHCO_3$) solution when compared to either tap water or Clorox bleach. Using a 10 mg/mL $NaHCO_3$ washing solution, it took 12 and 15 min to completely remove thiabendazole or phosmet surface residues, respectively, following a 24 h exposure to these pesticides, which were applied at a concentration of 125 ng/cm^2. LC−MS/MS results showed, however, that 20% of applied thiabendazole and 4.4% of applied phosmet had penetrated into the apples following the 24 h exposure. Thiabendazole, a systemic pesticide, penetrated 4-fold deeper into the apple peel than did phosmet, a non-systemic pesticide, which led to more thiabendazole residues inside the apples, which could not be washed away using the $NaHCO_3$ washing solution. This study gives us the information that the standard postharvest washing method using Clorox bleach solution for 2 min is not an effective means to completely remove pesticide residues on the surface of apples. The $NaHCO_3$ method is more effective in removing surface pesticide residues on apples. In the presence of $NaHCO_3$, thiabendazole and phosmet can degrade, which assists the physical removal force of washing. However, the $NaHCO_3$ method was not completely effective in removing residues that have penetrated into the apple peel. The overall effectiveness of the method to remove all pesticide residues diminished as pesticides penetrated deeper into the fruit. In practical application, washing apples with $NaHCO_3$ solution can reduce pesticides mostly from the surface. Peeling is more effective to remove the penetrated pesticides; however, bioactive compounds in the peels will become lost too.

KEYWORDS: *pesticides, washing, apple, SERS*

INTRODUCTION

The use of pesticides in agriculture has led to an increase in farm productivity.[1−3] However, pesticide residues may remain on agricultural produce, where they contribute to the total dietary intake of pesticides.[4−6] Concerns about potential hazards of pesticides to food safety and human health have increased, and therefore, it is desirable to reduce these residues.

Washing is the common practice to remove pesticide residues from produce. During the commercial processing of fresh produce, sanitizers are used in the postharvest washing process to remove visible soil or organic matter residues as well as to reduce the microbial contamination found on the surface.[7,8] One of the commercially available sanitizer washing solutions for produce is Clorox Germicidal bleach, which is approved by the United States Environmental Protection Agency (U.S. EPA) and is the most commonly used product. The active ingredient is sodium hypochlorite, and the solution pH is maintained at 6.5−7.5 with acetic acid. The U.S. EPA regulation (U.S. EPA regulation number 5813-100) for fruit and vegetable washing is that a sanitizing solution of 25 mg/L available chlorine is prepared and fruits or vegetables are submerged for 2 min, followed by a water rinse prior to packaging. Additionally, many methods have been examined on the removal of pesticide residues from fresh produce during home preparation.[9−11] Basically, we can use tap water or water containing various chemicals, such as sodium bicarbonate ($NaHCO_3$), also known as baking soda, sodium carbonate (Na_2CO_3), acetic acid (CH_3COOH), or sodium chloride (NaCl). The mechanisms for washing pesticides can be described as chemical and physical removal.[12] In the chemical way, pesticides can be partly decomposed in chemical solutions and the degradation products can be further removed by washing. Physical washing was achieved by removing pesticide directly. Pesticides can be washed away by the combination of chemical and physical forces.[13,14]

On the basis of our previous studies, pesticides that are applied to the produce surface can penetrate into the produce over time.[15−18] Systemic pesticides can penetrate more rapidly and deeper compared to non-systemic pesticides. The objective of this study was to investigate the effectiveness of commercial (Clorox Germicidal bleach) and homemade (tap water and

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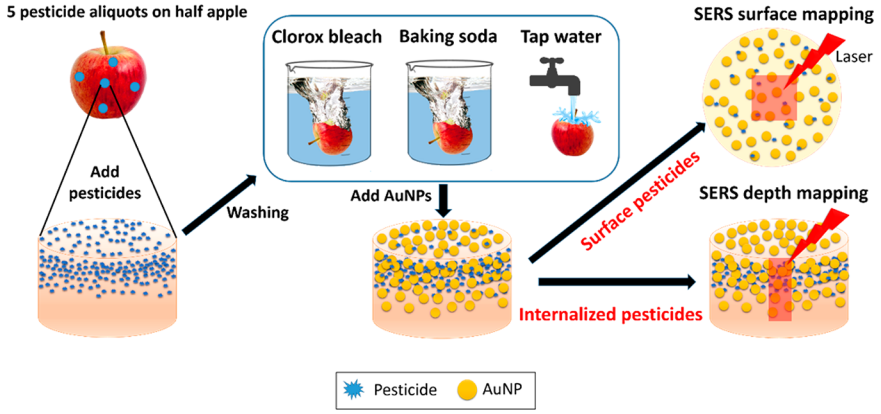
Scheme 1. Schematic Illustration of the SERS Mapping Method for Evaluation of Removal Effectiveness of Pesticide Residues on and in Apples with Commercial and Homemade Washing Agents[a]



[a]The SERS surface mapping image and depth mapping image are integrated with 25 and 75 scanning spots, respectively.

$NaHCO_3$) washing agents to remove surface and internalized pesticide residues on and in apples, respectively. We hypothesize that systemic pesticides will be more difficult to remove from whole apples as a result of their greater penetrating abilities. After application and washing, we monitored the amount of each pesticide or its degradation products that remained on the surface and penetrated into the apple over time using the surface-enhanced Raman spectroscopy (SERS) mapping method. If pesticides degraded to other molecules but were still left on the surface, SERS would be able to detect some signals. SERS techniques have been shown to be an effective tool to monitor pesticide residues on fresh produce, both on the surface and internally following penetration, using gold nanoparticles (AuNPs) as probes coupled with a confocal Raman instrument.[12,13] We have previously shown that AuNPs can penetrate fresh produce and interact with internalized pesticides, improving their Raman signals. A liquid chromatography tandem mass spectrometry (LC−MS/MS) method was developed to determine the penetration behavior of each pesticide and the effectiveness of the $NaHCO_3$ washing method in removing each pesticide.

To the best of our knowledge, this study is the first to use the SERS method to investigate the effectiveness of removing surface pesticide residues from apples following washing. Moreover, this work is the first to evaluate the removal of internalized pesticide residues from fresh produce. Understanding the effectiveness of various washing procedures in the removal of pesticides on and in apples will allow us to develop better strategies to minimize pesticide exposure from fresh produce.

MATERIALS AND METHODS

Materials. Thiabendazole [fungicide: 2-(4-thiazolyl)-1*H*-benzimidazole, ≥99%, analytical grade], ferbam [fungicide: iron(III) dimethyldithiocarbamate, ≥99%, analytical grade], and phosmet (insecticide: *O*,*O*-dimethyl *S*-phthalimidomethyl phosphorodithioate, ≥99%, analytical grade) were purchased from Sigma-Aldrich (St. Louis, MO, U.S.A.). Citrate-capped AuNP colloids with different diameters were purchased from Nanopartz, Inc. (Loveland, CO, U.S.A.). Sodium bicarbonate ($NaHCO_3$) was purchased from Fisher Scientific (Pittsburgh, PA, U.S.A.). Organic Gala apples and Clorox Germicidal bleach product with the active ingredient of 8.25% sodium hypochlorite, yielding 7.85% available chlorine, were purchased from Stop & Shop Supermarket (Hadley, MA, U.S.A.). All reagents were used without further purification. Ultrapure water (18.2 MΩ cm) was obtained using a Thermo Scientific Barnstead Smart2Pure water purification system (Waltham, MA, U.S.A.) and used for the preparation of all solutions.

Characterization of SERS Signals from Two Pesticides Applied to Apples. Gala apple was chosen as the test produce as a result of the wide range of pesticides used in its cultivation and because of its wide consumptions. Two pesticides, the systemic fungicide thiabendazole and the non-systemic insecticide phosmet, were chosen to be applied to the apple surface because thiabendazole is commonly used postharvest as a dip or spray and phosmet is used for control of a wide variety of pests.[19−23] According to the Food and Agriculture Organization of the United Nations (FAO), the waiting time of thiabendazole and phosmet is about 8−30 and 2−28 days, respectively. Over time, surface pesticides can be degraded by external environmental factors [e.g., ultraviolet (UV)] and internalized pesticides can be degraded by the plant enzyme biochemically.[24] The use of AuNPs as SERS substrates was critical in the detection of pesticide residues and, thus, in the evaluation of the removal effectiveness of different washing agents. The size of AuNPs was important for both signal intensity and penetration depth. Therefore, different sizes of AuNPs (15, 30, 50, 70, 90, and 125 nm) as SERS substrates were first evaluated. Ferbam was used as a Raman indicator to monitor AuNP penetration in this study as a result of the strong SERS activities of ferbam, thus facilitating the track of AuNP penetration. In addition, our previous study shows that 20 mg/L ferbam does not penetrate into apples following a 30 min exposure and, therefore, does not influence AuNP penetration.[16] In detail, a 1 mL aliquot of a 20 mg/L ferbam solution was mixed with a 1 mL aliquot of a 250 mg/L AuNP solution for 30 min to form a ferbam/AuNP complex through the Au−amino bond. A 5 μL aliquot of the mixture was pipetted onto apples. After air drying for 30 min, SERS depth mapping images were obtained using a confocal Raman instrument. The AuNPs with the deepest penetration depth were selected as the best probe for the following studies.

Thiabendazole and phosmet stock solutions of 1000 mg/L (ppm) were prepared with ultrapure water and methanol (1:1, v/v) and then diluted to 100 mg/L with ultrapure water before use. A 50 μL aliquot of each pesticide solution was mixed with 50 μL of a 250 mg/L solution of 50 nm AuNPs for 1 h at room temperature to ensure effective pesticide complexation with AuNPs through the Au−thiol or Au−amino bond. Apple peels with a surface area of ∼8 cm^2 and thickness of about 0.5 cm were prepared using a sharp knife to cut from a whole apple. Then, a 5 μL aliquot of each of the pre-prepared pesticide/AuNPs solutions was pipetted onto each apple peel with a concentration of about 125 ng/cm^2 situated on a glass slide and air-dried in a fume hood for 10 min. Solutions of AuNPs without pesticide and each pesticide alone were also pipetted onto apples as control treatments for comparison purposes.

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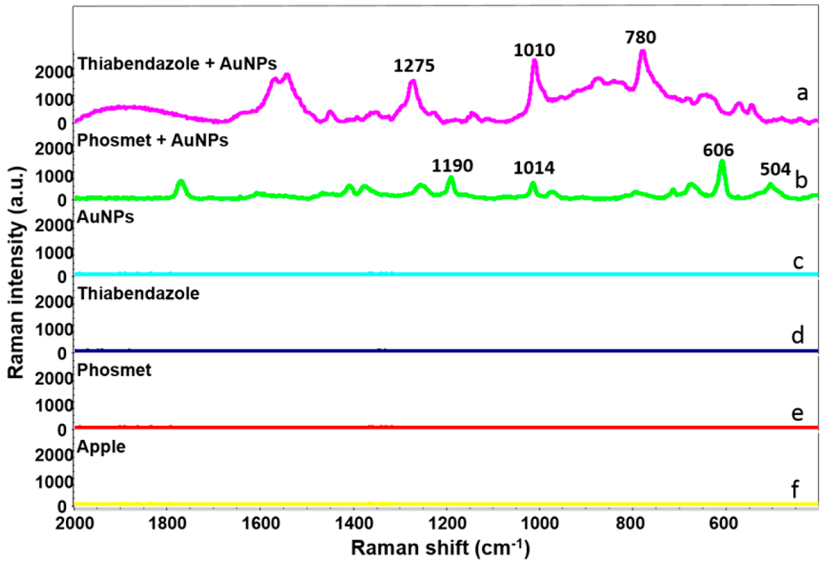

Figure 1. (A) Raman spectra on apples: (a) thiabendazole with AuNPs, (b) phosmet with AuNPs, (c) AuNPs, (d) thiabendazole, (e) phosmet, and (f) apple.

Removal of Surface Pesticide Residues by Washing. Different washing methods were investigated to study their effectiveness in removing surface pesticide residues from apples. Organic apples were thoroughly hand rinsed with ultrapure water for 2 min and air-dried before the experiment to remove surface contaminants, such as dust. A schematic illustration (Scheme 1) shows the washing protocol used for the study of the removal of surface pesticide residues from apples. First, 10 aliquots (5 μL) of a 100 mg/L solution of each pesticide were pipetted onto the surface of an apple (5 aliquots per half apple), each with an approximate concentration of 125 ng/cm^2. After the aliquots were air-dried on the apples for 30 min, three different washing agents/methods were investigated: Clorox Germicidal bleach (25 mg/L available chlorine, pH 8.05), 10 mg/mL $NaHCO_3$ (pH 9.12), and tap water (pH 6.85). For Clorox and $NaHCO_3$ solutions, the treated apples were first immersed into 200 mL of a washing solution for 2 or 8 min and then gently rinsed with 150 mL of deionized water for 10 s. The tap water washing method, which was intended to imitate how people washed their apples at home, was applied by rinsing treated apples with approximately 1.8 L of tap water for 2 min at a flow rate of 15 mL/s. All washed apples were air-dried at room temperature for 10 min. The intact apple was then cut vertically into two halves, with each half apple having 5 aliquots of the treatment pesticide applied before. One half of the apple was used for SERS detection and the other half was used for LC−MS/MS detection. For the SERS detection of surface pesticide residues, a 5 μL aliquot of a 250 mg/L AuNP solution was pipetted onto the same position where the pesticide had been previously applied using a camera to record the position. After air drying the AuNP solution for 30 min, apple peels (approximately 8 cm^2 area and 0.5 cm thickness) with 1 aliquot (5 μL) of a 100 mg/L solution of each pesticide in the center of the 8 cm^2 area were cut from each treated apple using a sharp knife and then SERS surface mapping was performed on that area to evaluate washing effectiveness. The detail of aliquots of the pesticide on apples is shown in Figure S1 of the Supporting Information. Pesticide-treated apples that were not washed and apples with only AuNPs (no pesticides) were also analyzed using the SERS surface mapping method for comparison. For each apple sample, the corresponding SERS depth mappings were also collected. Each treatment was repeated 3 times.

Removal of Internalized Pesticide Residues. SERS depth mapping methods were applied to apples to evaluate the effectiveness of different washing agents for removing internalized pesticides that increased over exposure time (Scheme 1). The washing treatments were Clorox Germicidal bleach (25 ppm available chlorine), 10 mg/mL $NaHCO_3$ solution, and tap water. Organic apples were also first washed to remove surface contaminants and then treated by applying each pesticide solution as described for the study of surface pesticide residues above. Apple samples were then air-dried at room temperature over different time intervals (30 min and 24 h) to allow for pesticide penetration into apples. Because pesticide residues still remained on the surface, these residues were needed to be removed completely before the effectiveness of different washing methods could be compared in their ability to remove internalized pesticides. $NaHCO_3$ solution (10 mg/mL) was used to remove surface thiabendazole and phosmet residues by rinsing apples for 12 and 15 min, respectively. After that, three different washing treatments were examined: (1) Clorox Germicidal bleach (25 mg/L available chlorine) applied for 8 min, (2) 10 mg/mL $NaHCO_3$ solution applied for 8 min, and (3) tap water applied for 2 min. The washing process is as follows. For Clorox and $NaHCO_3$ solutions, the treated apples were first immersed into 200 mL of a washing solution for 8 min and then gently rinsed with 150 mL of deionized water for 10 s. The tap water washing method was applied by rinsing treated apples with approximately 1.8 L of tap water for 2 min at a flow rate of 15 mL/s. After apple samples were air-dried at room temperature, a 5 μL aliquot of the 250 mg/L AuNP solution was added at the same position where pesticides were applied using the same protocol used in the study of surface residues described above. After 30 min of air drying, SERS depth mapping images were obtained using the confocal Raman instrument. SERS depth mapping images of apple samples with the only removal of surface pesticide residues were also collected for comparisons. Each treatment was repeated 3 times.

Raman Instrumentation and Data Analysis. A DXR Raman microscope (Thermo Fisher Scientific, Madison, WI, U.S.A.) with a 780 nm laser and a 20× long distance microscope objective was used in this study. Each sample was scanned from 400 to 2000 cm^{-1} for a 2 s exposure time. To measure pesticides on apple surfaces, Raman spectra and SERS surface mapping images with an area of 100 × 100 μm were carried out using a 50 μm slit aperture and 5 mW laser power to maximize the signals. The step size of the surface mapping was 20 μm, and one image contained the data from 25 scans from the area where the pesticide aliquot was applied. The SERS spectra obtained from multiple spots of the same sample were averaged and analyzed with TQ Analyst (version 8.0). For penetration studies, SERS depth mapping images were obtained using a 50 μm pinhole aperture and 5 mW laser power to control the confocal depths using a scanning depth of 300 μm. Each depth scanning was collected from the cross-section vertical to the apple surface with an area of 100 × 300 μm and depth of 300 μm. The step size of the depth mapping was 20 μm, and one image contained the data from 75 scanning spots. The SERS spectra of 75 spots can integrate to generate artificial color images based on the

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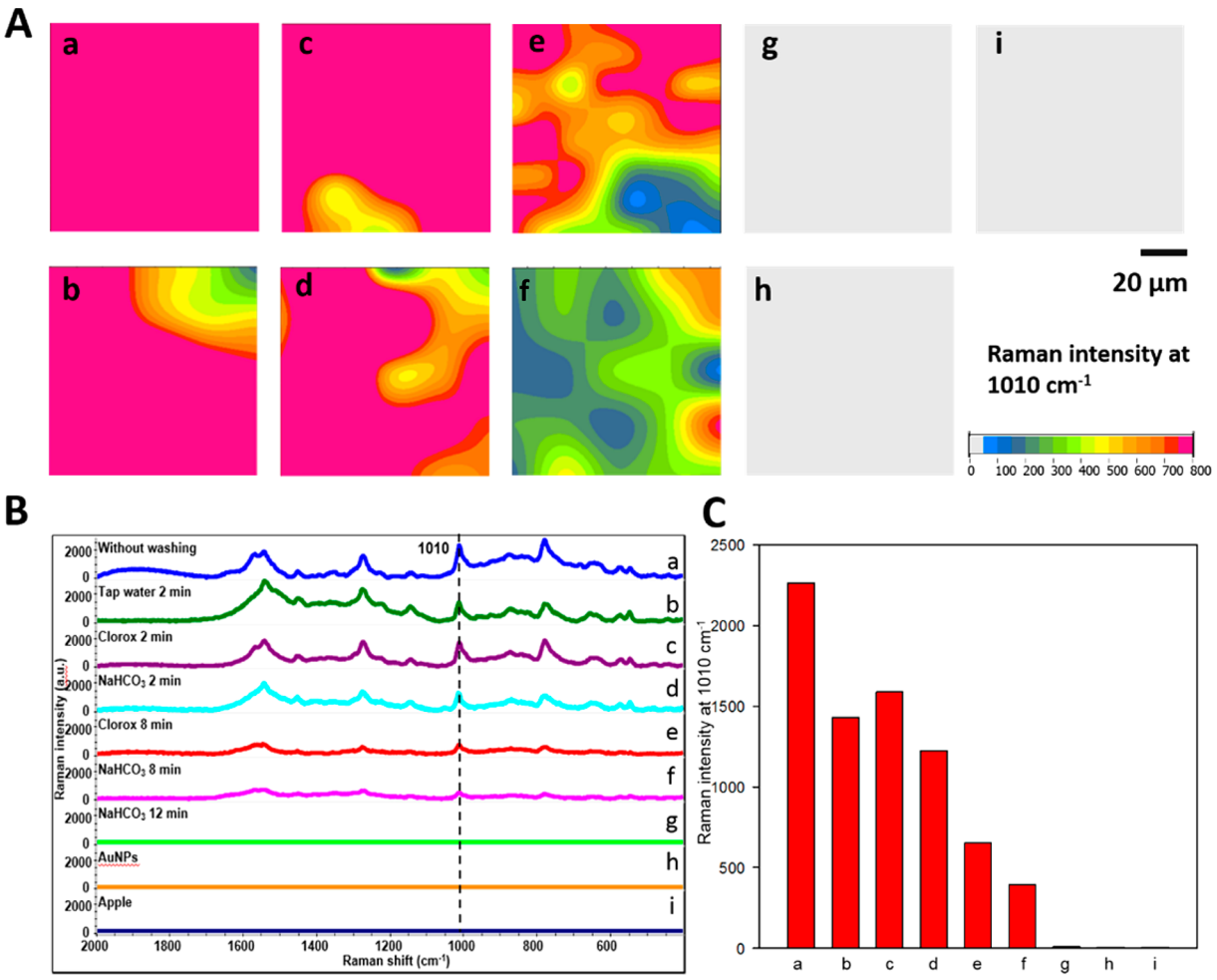

Figure 2. (A) SERS surface mapping images of the apple surface with thiabendazole after different washing treatments: (a) without washing, (b) tap water washing for 2 min, (c) Clorox solution washing for 2 min, (d) $NaHCO_3$ solution washing for washing 2 min, (e) Clorox solution washing for 8 min, (f) $NaHCO_3$ solution washing for washing 8 min, (g) $NaHCO_3$ solution washing for washing 12 min, (h) AuNPs on the apple surface, and (i) apple surface alone for comparisons. The step size is 20 μm, and one image contains 25 scanning points. (B) Corresponding SERS average spectra of each mapping image. (C) Corresponding Raman intensities in each SERS spectrum.

intensity of a designated Raman peak. Raman images were based on the characteristic peaks in the pesticide spectra using the Atlμs Function in the OMNIC software (Thermo Fisher Scientific).

LC–MS/MS. The apple samples were also analyzed with the LC–MS/MS method. The limits of detection of LC–MS/MS analysis of phosmet and thiabendazole are both 1 μg/L. The pesticides on and in apple samples were first extracted by the QuEChERS standard operating procedure based on a published study[25] and the Agilent Application Notebook. Extracts were measured on Waters Alliance LC equipped with a Waters Acquity TQD MS/MS system at the Massachusetts Pesticide Analysis Laboratory. The analytical column was Atlantis T3, 2.1 × 100 mm, maintained at 30 °C. Mobile phases consisted of 0.1% formic acid/water (phase A) and 0.1% formic acid/acetonitrile (phase B). We started with 95:5 A/B, held for 0.5 min, ramped to 95% B at 7 min, held until 12 min, ramped to 95% A at 13 min, and held until 18 min to equilibrate. The flow rate was kept at 0.2 mL/min, and the injection volume was 10 μL. Capillary voltage was set at 3000 V. High-purity argon (99.999%) was used as collision gas. The ion source temperature was 250 °C, with nitrogen for desolvation. Chromatograms were obtained in the positive ion and multiple reaction monitoring (MRM) mode. MRM conditions: positive ionization ES+, with collision gas of 0.2 mL/min. For thiabendazole, retention time, 7.85 min; parent ion, 201.96; quantifying ion, 65; and qualifying ion, 131. For phosmet, retention time, 11.70 min; parent ion, 318; quantifying ion, 160; and qualifying ion, 133.

RESULTS AND DISCUSSION

Characterization of SERS Spectra from Pesticides Applied to Apples. Citrate-capped AuNPs (50 nm), which can be detected to a depth of approximately 220 μm in apple peel, were chosen as the probe for detection of penetrated pesticides (Figure S2 of the Supporting Information). The characteristic SERS peaks for either thiabendazole or phosmet enhanced by AuNPs are shown in Figure 1. SERS fingerprint information for each pesticide in the presence of AuNPs is clearly observed, and the peaks at 1010 cm^{-1} for thiabendazole and 606 cm^{-1} for phosmet were chosen as the characteristic peaks for SERS mapping. The characteristic peaks for either thiabendazole or phosmet were substantially reduced or absent from SERS spectra when AuNPs were absent or from apple samples that received no pesticide or AuNP treatments. The concentration-dependent SERS spectra for either thiabendazole or phosmet on apples were studied in our previous published research, where thiabendazole could be detected as low as 2 μg/L and phosmet could be detected as low as 10 μg/L, showing the ultrahigh sensitivity of the developed SERS method for the detection of pesticides on apples.[16]

Effectiveness of Washing Methods on the Removal of Surface Pesticide Residues. SERS mapping methods were

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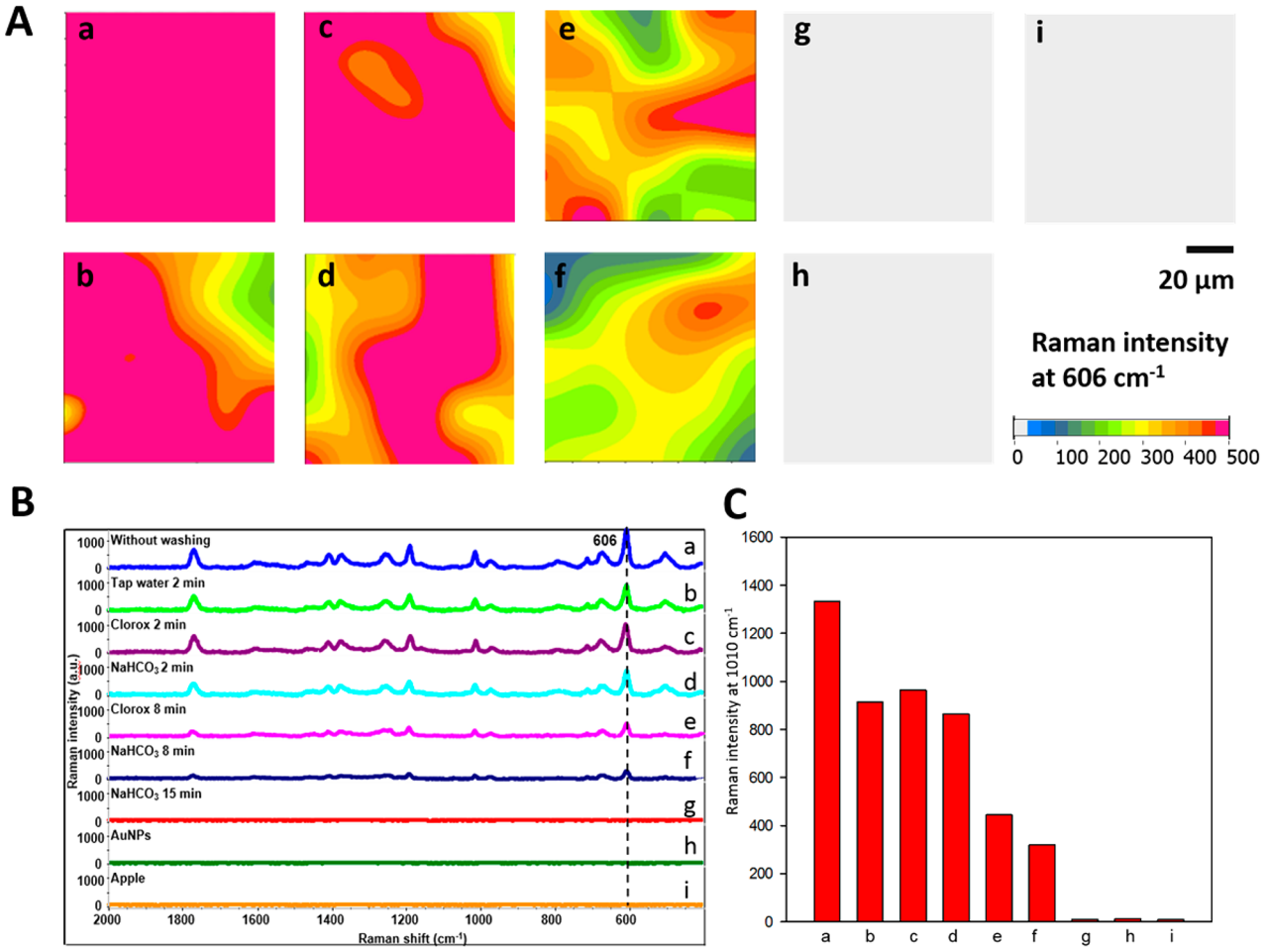

Figure 3. (A) SERS surface mapping images of the apple surface with phosmet after different washing treatments: (a) without washing, (b) tap water washing for 2 min, (c) Clorox solution washing for 2 min, (d) $NaHCO_3$ solution washing for 2 min, (e) Clorox solution washing for 8 min, (f) $NaHCO_3$ solution washing for 8 min, (g) $NaHCO_3$ solution washing for 15 min, (h) AuNPs on the apple surface, and (i) apple surface alone for comparisons. The step size is 20 μm, and one image contains 25 scanning points. (B) Corresponding SERS average spectra of each mapping image. (C) Corresponding Raman intensities in each SERS spectrum.

used to evaluate the effectiveness of different washing agents/methods in removing the surface residues of the systemic pesticide thiabendazole (panels a–g of Figure 2A). Each SERS surface mapping image was integrated at 25 locations within each pesticide applied area and gives final artificial color images. The rose color indicates pesticides of higher signal intensity and, thus, higher concentrations of pesticides, while the white color means pesticides were not detected. For comparison purposes, SERS surface mapping images were obtained from apples that (1) received thiabendazole treatment and AuNPs but were not washed (panel a of Figure 2A), (2) received AuNPs but no thiabendazole (panel h of Figure 2A), and (3) received no treatment (panel i of Figure 2A). The SERS spectra collected from 25 locations in each mapping image were averaged, and final SERS spectra are shown in Figure 2B. Figure 2C gives the corresponding Raman intensity of characteristic peaks of thiabendazole at 1010 cm^{-1}. It is clear that, after washing, the amount of thiabendazole on the surface of the apple decreased. After 2 min of washing with different methods (panels b–d of Figure 2A), $NaHCO_3$ solution resulted in the greatest pesticide loss when compared to either tap water or Clorox solutions. This is because, in the presence of $NaHCO_3$, thiabendazole and phosmet can degrade, which assists the physical removal force of washing. From the LC–MS/MS study, we found 51% thiabendazole degraded in 12 min and more than 95.8% phosmet degraded in 15 min in 10 mg/mL $NaHCO_3$ washing solution. In addition, thiabendazole does not interact well with the epicuticle of apples as a result of the formation of the higher surface tension of the droplets, which has reduced spreading properties on fruit surfaces.[21] Therefore, thiabendazole would be easily rinsed off of the fruit. When the washing time was increased to 8 min (panels e and f of Figure 2A), there was a substantial decrease in the surface residues of thiabendazole and the $NaHCO_3$ solution was again the most effective treatment. Upon increasing the $NaHCO_3$ solution washing time to 12 min (panel g of Figure 2A), the SERS signals from surface thiabendazole residues were not detected, which, given the detection limit of the SERS method, meant that the surface residues were negligible. Apples treated with only AuNPs and apples without AuNPs or thiabendazole did not show any characteristic SERS peaks (panels h and i of Figure 2A). These results indicated that increasing washing times resulted in the increased removal of surface thiabendazole residues. The longer washing times caused more loss of surface pesticides. The result also indicated that the standard postharvest washing method with Clorox bleach solution for 2 min did not effectively remove surface thiabendazole.

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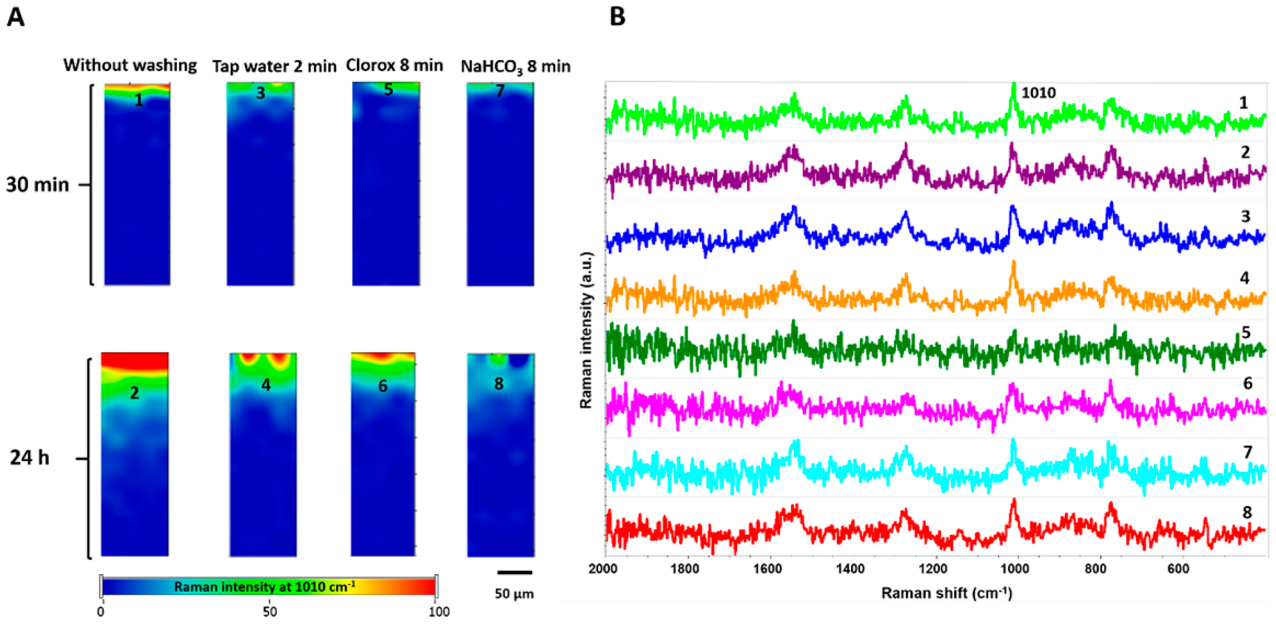

Figure 4. (A) SERS depth mapping images of internalized thiabendazole after different washing conditions following different exposure time periods. The step size is 20 μm, and one image contains 75 scanning points. (B) SERS spectra of selected positions on the mapping images.

The apples were also analyzed using the SERS depth mapping method (Figure S3 of the Supporting Information). Overall, this analysis showed that thiabendazole was able to penetrate into the apple peel following the 2 min wash and the amount of residue decreased following the 8 min washing (panels b–g of Figure S3 of the Supporting Information). The internalization of thiabendazole is likely to be the result of two processes: (1) penetration by the pesticide itself and (2) penetration of the pesticide complexed with AuNPs. After washing with $NaHCO_3$ solution for 12 min, all of the remaining thiabendazole residues are due to internalized residues because surface thiabendazole residues were reduced below the detection limit by this method (panel g of Figure S3 of the Supporting Information). Therefore, even though surface residues can be removed, there was still substantial amounts of thiabendazole in the apple peel, some of which were from the direct penetration of thiabendazole itself. Given this finding, there appears to be a critical need to study the effective removal of internalized pesticides.

Similar results were obtained using the non-systemic pesticide, phosmet (Figure 3). Overall, $NaHCO_3$ solution was more efficient than tap water and Clorox solution. This result also indicated that the Clorox-based commercial postharvest washing method was not an effective method to remove phosmet from treated apples, in that only a small amount of phosmet was removed. When the washing time was increased to 8 min (panels e and f of Figure 3A), there was an obvious reduction of surface phosmet residues and the $NaHCO_3$ solution was more effective than the Clorox solution. Surface phosmet residues could be essentially removed by further increasing the washing time of the $NaHCO_3$ method to 15 min (panel g of Figure 3A). As with thiabendazole, surface phosmet residues were increasingly removed by increasing the washing times.

Phosmet-treated apples were also analyzed using the SERS depth mapping method (Figure S4 of the Supporting Information). After different washing treatments (panels b–g of Figure S4 of the Supporting Information), internalized phosmet residues were likewise found in apples. When surface phosmet residues were essentially removed using the $NaHCO_3$ solution and washing for 15 min, SERS depth mapping images still detected phosmet residues, which meant that phosmet, a non-systemic pesticide, can still penetrate into apples (panel g of Figure S4 of the Supporting Information).

Effectiveness of Washing on the Removal of Internalized Pesticide Residues. SERS depth mapping methods were also applied to determine the effectiveness of different washing agents/methods in removing internalized pesticides over time. Surface pesticide residues must be completely removed before the addition of AuNP probes because any contaminating surface pesticide residue would penetrate into apples as AuNP/pesticide complexes, which will form on the apple surface. Therefore, it is necessary to remove contaminating surface pesticide residues to study the removal of internalized pesticides. $NaHCO_3$ solution (10 mg/mL) was applied to remove surface thiabendazole and phosmet residues using washing times of 12 and 15 min, respectively, based on the above surface residue removal study. After removal of contaminating surface residues, three washing methods were used: 10 mg/mL $NaHCO_3$ solution with washing for 8 min, Clorox solution with washing for 8 min, and tap water with washing for 2 min. The SERS depth mapping images for the penetration of thiabendazole following different exposure times (30 min and 24 h) before removing internalized pesticide (no washing) and after attempting to remove internalized pesticide (after washing) are shown in Figure 4A. The red color indicates pesticides of higher signal intensity, while the blue color means pesticides were not detected. Without the removal of internalized thiabendazole, SERS depth mapping images indicate that thiabendazole penetrated to a depth of 30 μm following the 30 min exposure, and this depth increased to approximately 80 μm when the exposure time was increased to 24 h. When tap water was applied as the washing solution for 2 min, there was no obvious decrease in the penetration depth for thiabendazole compared to the no wash method but the amount of thiabendazole close to the apple surface was reduced

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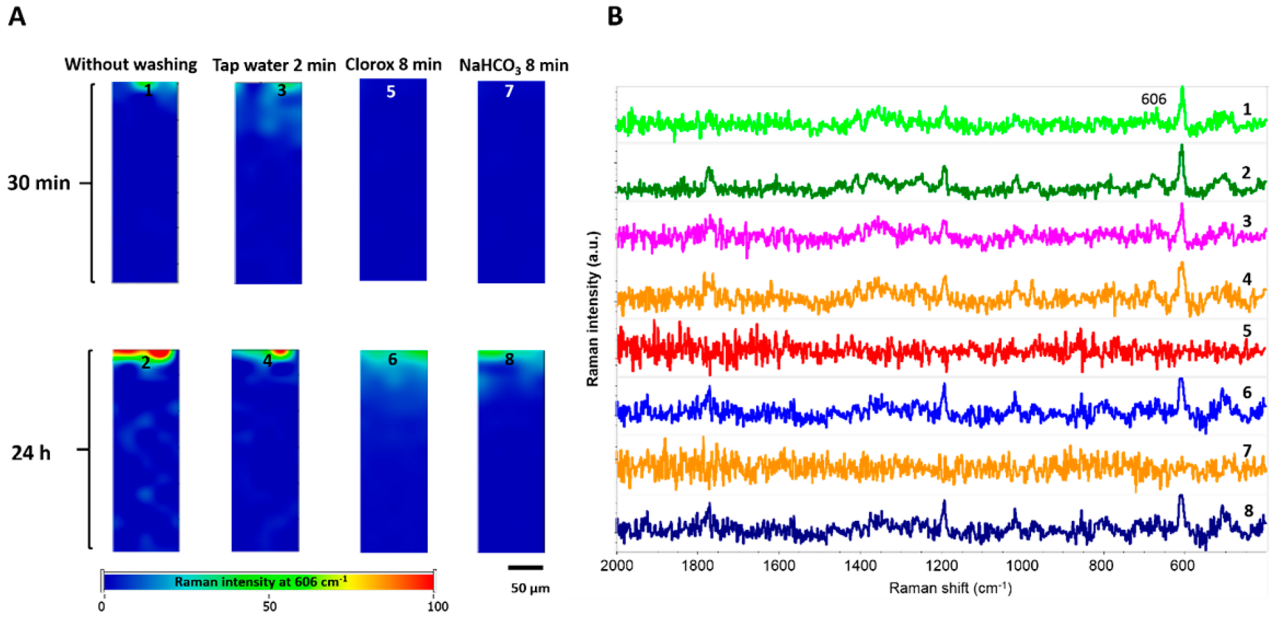

Figure 5. (A) SERS depth mapping images of the internalized phosmet after different washing conditions following different exposure time periods. The step size is 20 μm, and one image contains 75 scanning points. (B) SERS spectra of selected positions on the mapping images.

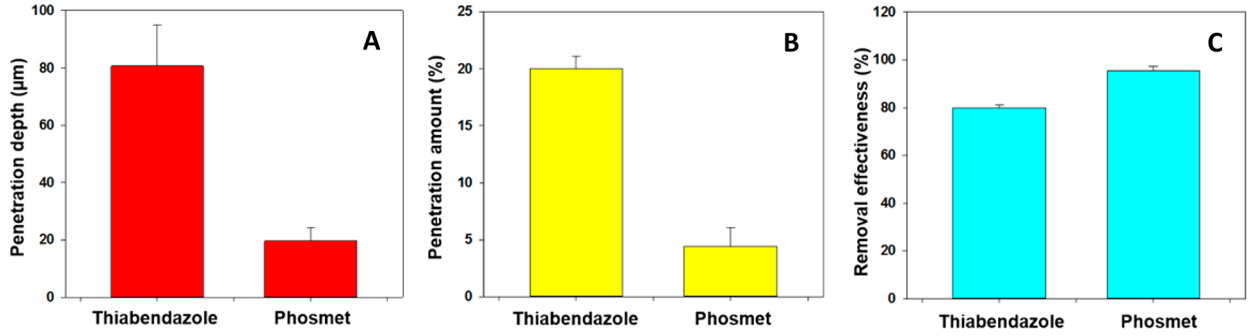

Figure 6. (A) Penetration depths and (B) penetration amounts of thiabendazole and phosmet and (C) removal effectiveness by 10 mg/mL $NaHCO_3$ solution washing after a 24 h exposure.

at both the 30 min and 24 h exposure times. After washing with either the $NaHCO_3$ or Clorox solutions for 8 min, the penetration depths for thiabendazole decreased slightly following exposures of 30 min and 24 h, respectively, compared to the no wash method. The thiabendazole residues close to the apple surface were also reduced. The corresponding SERS spectra of positions 1−8 from the SERS mapping images given in Figure 4B clearly show the diagnostic fingerprint of thiabendazole.

On the basis of a published study,[26] when a pesticide solution was applied on the surface of apples, it was first absorbed onto the surface of epicuticular wax and then diffused into the wax and cuticle. Equilibrium of the pesticide on the epicuticular wax and the pesticide in the wax and cuticle is established when the amounts of pesticides in wax and cuticle no longer increase. Once equilibrium is achieved, pesticides begin to penetrate into the living cells below, and this fraction of the pesticide is retained irreversibly. When we applied washing treatments on apples, pesticides that had penetrated into the wax and cuticle moved back to the surface of the epicuticular wax until equilibrium was re-established and were then removed by the washing treatment. Therefore, the amount of thiabendazole close to the surface was reduced, and there was a slight decrease of the penetration depth because of the upward movement during the re-establishment of equilibrium. It is noteworthy that the thickness of the wax and cuticle of apples varied from 30 to 75 μm. Because thiabendazole was shown to penetrate to a depth of 80 μm after a 24 h exposure, it may have penetrated into the living cells.[27−30] If true, it would be very difficult to remove pesticides by washing once they enter cells as a result of the irreversible binding process there.

The removal of internalized phosmet residues was studied using the same methods and analysis used for thiabendazole described above (Figure 5A). Overall, phosmet, a non-systemic pesticide, still penetrated into apples. With increased exposure times, the penetration depth of phosmet increased to approximately 20 μm after 24 h in unwashed apples. There was little change in the penetration depth after washing in tap water for 2 min. Using an 8 min wash with either $NaHCO_3$ or Clorox solutions, no phosmet was detected following a 30 min exposure, which meant all phosmet residues were removed during the wash, indicating that phosmet only penetrated into the wax and cuticle layers of apples during the 30 min exposure. Using an 8 min wash with either $NaHCO_3$ or Clorox solutions, there were substantial amounts of phosmet present following a 24 h exposure but no apparent change in the penetration depth was seen compared to unwashed apples. Nevertheless, the amount of phosmet close to the surface of the apple was

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reduced. The corresponding SERS spectra of positions 1−8 from the SERS mapping images given in Figure 5B clearly show the characteristic peaks of phosmet.

LC−MS/MS Analysis. LC−MS/MS was used to determine the amount of each pesticide as well as the effectiveness of a 10 mg/mL $NaHCO_3$ solution in removing thiabendazole residues from apples during a 12 min wash and phosmet residues during a 15 min wash following a 24 h exposure for both pesticides. Under these washing conditions, surface pesticide residues were reduced to below the detection limit for each pesticide. Thus, the amount of pesticide detected was from internalized pesticides only. The relationship between the penetration depth (A) and amount of each pesticide that penetrated the apple (B) and the effectiveness of 10 mg/mL $NaHCO_3$ solution in removing each pesticide (C) are shown in Figure 6. These data demonstrated that, after a 24 h exposure, thiabendazole penetrated to a depth of 80 μm compared to phosmet that penetrated only to a depth of 20 μm (Figure 6A). From the amounts of each pesticide that penetrated, 20% of applied thiabendazole and 4.4% of applied phosmet penetrated into apples (Figure 6B). Thus, the overall effectiveness of the wash method used to remove thiabendazole and phosmet residues from whole apples was determined to be 80 and 95.6%, respectively (Figure 6C). These results indicated that the systemic pesticide, thiabendazole, penetrated deeper than the non-systemic pesticide, phosmet. Furthermore, the deeper the penetration depth as seen with thiabendazole, the higher the level of the internalized pesticide, indicating that the wash method used to remove internalized pesticide residues was not complete. In addition, LC−MS/MS was applied to evaluate the amounts of pesticides in the $NaHCO_3$ washing solution. The results showed that 49% of thiabedozale was detected in the washing solution, but no phosmet was detected in the washing solution because the phosmet molecules degraded very fast.

In conclusion, we investigated the effectiveness of commercial and homemade washing methods in removing both systemic (thiabendazole) and non-systemic (phosmet) pesticides from the surface of and inside apples using SERS mapping and LC−MS/MS methods. The results showed that the 10 mg/mL $NaHCO_3$ solution was most effective in removing thiabendazole and phosmet on and in apples, whereas the standard postharvest washing method with Clorox bleach solution and a 2 min wash did not effectively remove these pesticides. We determined that 20% of applied thiabendazole and 4.4% of applied phosmet penetrated into apples after a 24 h exposure, giving an overall removal efficiency of 80% for thiabendzole and 95.6% for phosmet using the 10 mg/mL $NaHCO_3$ solution and washing for 12 and 15 min, respectively. This result showed that the systemic pesticide, thiabendazole, which penetrated deeper, was more difficult to remove compared to the non-systemic pesticide, phosmet, because internalized pesticides that penetrate into the cells below the waxy and cuticle layer of the apple are irreversibly bound there. For apples, the peel can easily be removed along with most of the internalized pesticide residues; however, important nutrients (e.g., polyphenolic compounds, fibers, pigments, vitamins, and minerals) will also be lost.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jafc.7b03118.

Schematic illustration of pesticide on apples (Figure S1), SERS depth mapping images of different sizes of the AuNP/ferbam complex penetrated into apples using the intensity of the SERS peak of ferbam at 1373 cm^{-1} and SERS spectra of selected positions on the mapping images (Figure S2), SERS depth mapping images of apples with thiabendazole after different washing treatments (Figure S3), and SERS depth mapping images of apples with phosmet after different washing treatments (Figure S4) (PDF)

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Notes

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