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Article

Antibacterial activity and absorption of paper towels made from fruit peel extracts

Prarthana Prashanth[1], Srikant Lokesh[2], Milind Sagaram[3], Nathan B. Pincus[4], Alan R. Hauser[4]

[1] Illinois Mathematics and Science Academy, Aurora, Illinois
[2] Waubonsie Valley High School, Aurora, Illinois
[3] Metea Valley High School, Aurora, Illinois
[4] Northwestern University Feinberg School of Medicine, Chicago, Illinois

SUMMARY

Barriers to adequate hygiene are important problems throughout the world, as billions of people do not have access to sanitary conditions. In particular, unsatisfactory hand hygiene leads to the spread of bacterial infections from person to person. To address this problem, we developed the PeelTowel, an antibacterial and water-absorbing towel made of a combination of fruit peels and recycled paper waste, which has the potential to make hand-hygiene accessible, sustainable, and environmentally friendly. Kiwi, orange, and lime peels were chosen for this purpose because they contain antibacterial factors such as vitamin C and citric acid as well as water-absorbing cellulose. PeelTowels were produced by creating a paste of crushed fruit peels and paper and then drying thin films of this paste on screens. PeelTowels were tested for their ability to inhibit the growth of bacteria and absorb water. They were incubated with *Escherichia coli*, and bacterial survival was measured by counting colonies on agar plates. Similarly, absorption was quantified by exposing PeelTowels to varying amounts of water. The Lime PeelTowel had the highest antimicrobial activity. It eradicated 50-91% of *E. coli* after exposure for 1 hour and 95-98% after exposure for 18 hours. It also absorbed three times the amount of water as a commercially available paper towel. Our results suggest that Lime PeelTowels have the potential to be an environmentally friendly option for antibacterial and absorptive hand towels.

INTRODUCTION

According to the World Health Organization, around 2.5 billion people (35% of the world's population) do not have access to adequate hygiene, including clean water, sanitizers, and other sanitation products (1). Educational campaigns have been undertaken to improve hygiene in the home, but people in developing nations frequently cannot afford soap or other basic products to maintain good health (2). The resulting lack of hygiene may lead to the spread of infections. Our goal was to develop a clean, eco-friendly, and easy to use paper towel product that has antibacterial activity.

The use of currently available paper towels creates several environmental challenges. Every year in the U.S. alone, 2 billion trees are harvested to make 85,000,000 tons of paper, and the average American uses 680 pounds of paper annually (3). Coincidentally, this usage is mirrored by consumption of fruit. The American Institute of Physics found that 15.6 million tons of citrus peel waste are created annually around the world (4). This waste makes its way to landfills and adds to the growing accumulation of discarded materials in the environment. These statistics highlight the need for new methods to improve hygiene while reducing and reusing waste. In particular, in areas where effective hygiene options are limited or inaccessible, we asked how an antibacterial hygiene product could be created that reduces paper and peel waste.

The peels of orange, kiwi, and lime contain antibacterial factors such as vitamin C, citric acid, flavonoids, and phenolic compounds (**Table 1**; 14-15). These factors work in different ways to kill bacteria. For example, vitamin C facilitates the killing of bacteria by the Fenton Reaction, in which reactive oxygen species are produced that are lethal to bacteria. In the Fenton reaction, ferrous iron reacts with hydrogen peroxide to generate ferric iron and antibacterial reactive oxygen species. Vitamin C aids in this process by converting ferric iron back to ferrous iron, thus allowing the Fenton Reaction to continuously produce reactive oxygen species. As an example of the importance of this reaction, the bacterium *Mycobacterium tuberculosis* is killed by vitamin C due to the reactive oxygen species that are produced (5). In addition to vitamin C, citric acid is also effective in killing bacteria. Citric acid may acidify the bacterium's environment, leading to inhibition of bacterial replication (6). Oranges, kiwis, and limes all contain high amounts of vitamin C, citric acid, flavonoids, and phenolic compounds, suggesting that they have antibacterial properties (7-14).

	Vitamin C (mg per 100 g)	Citric acid in juice (mg per 100 g)	pH	Cellulose content (%)
Orange	58.30	452	3.1 - 3.96	15
Lime	27.78	4124	4.35	14
Kiwi	92.72	1402	2.4	6

Table 1: Properties of orange, lime, and kiwi peels. The vitamin C and citric acid concentrations, pH, and cellulose contents of orange, lime, and kiwi peels (7, 10-14, 16, 17).

Many fruits also contain cellulose, which is highly effective in attracting and absorbing water (15). For this reason, cellulose from wood products is a major component of paper towels. Orange peels, lime peels, and kiwi contain 15%, 14%, and 6% cellulose, respectively (**Table 1**; 16, 17). These values suggest that extracts of orange, kiwi, and lime peels are capable of absorbing water under appropriate conditions.

Many of the infections that result from poor hand-hygiene are gastrointestinal in nature and manifest as diarrhea. For example, *Escherichia coli* is a Gram-negative bacterium normally found as a commensal in the intestines. However, while most strains of *E. coli* are harmless and even beneficial to the gut, pathogenic strains can cause infectious diarrhea. Infectious diarrhea occurs following ingestion of food or water contaminated by fecal material from an infected person or animal. For these reasons, good hand hygiene (e.g. the thorough washing of hands with soap or another disinfectant) can prevent many cases of illness caused by *E. coli* (18). Several other bacterial pathogens, such as *Salmonella enterica* and *Shigella* species are transmitted in a similar way.

The bactericidal and water-absorbing properties of orange, kiwi, and lime peels led us to hypothesize that peels from these fruits could be used with discarded paper waste to produce antibacterial paper towels. In the current study, we performed proof-of-concept tests to demonstrate that certain formulations of paper towels made of fruit peels and recycled paper (designated "PeelTowels") were water absorbent and killed *E. coli* bacteria.

RESULTS

Fruit peels and paper are used to generate PeelTowels

Our goal was to develop a simple, natural, and environmentally-friendly product for improved hand-hygiene, which we called the PeelTowel. Our prototype PeelTowel reused discarded fruit peels and paper and took advantage of the natural antimicrobial properties of fruit peels. Briefly, Orange PeelTowels, Lime PeelTowels, and Kiwi PeelTowels were constructed as follows: The three fruits were washed and peeled, and each peel was emulsified in a blender along with shredded paper and water. The contents of the blender were added to a handmade mold constructed from photo frames and window screens, which drained excess water from the PeelTowel. The PeelTowel was then dried with a cloth, sponge, and dryer. In this way, PeelTowels made from fruit peels and paper were produced for subsequent experiments (**Figure 1**).

PeelTowels demonstrate antibacterial activity following one hour of exposure

To test the antibacterial properties of PeelTowels and fruit peels compared to commercial paper towels, we generated a standard bacterial inoculum of known size. The bacterial concentration of *E. coli* was adjusted to reach approximately 10^8 colony forming units (CFU) per mL – the initial inoculum.

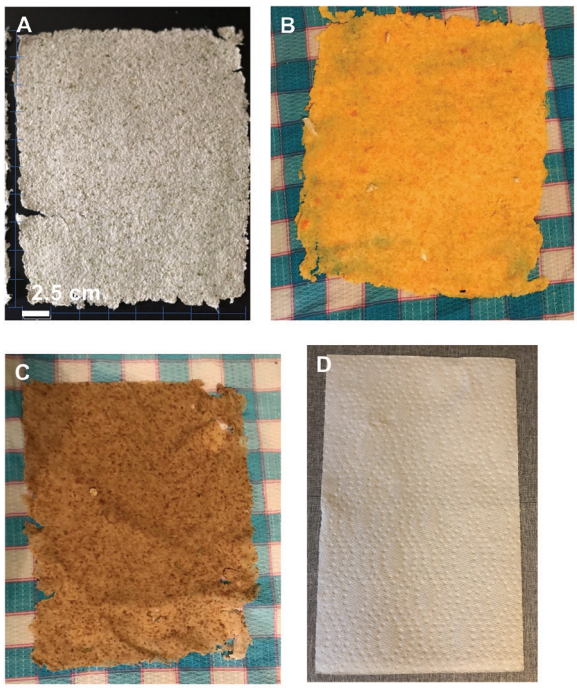

Figure 1: PeelTowels. Photos of **(A)** Lime PeelTowel with scale, **(B)** Orange PeelTowel, **(C)** Kiwi PeelTowel, and **(D)** paper towel.

The actual number of bacteria in the inoculum was then determined by streaking the inoculum on agar plates and counting colonies that had grown by following day. Based on these results it was estimated that a 10-µL inoculum contained 773,000 CFU of *E. coli*. This value was used in subsequent calculations.

To determine whether PeelTowels, fruit peels, and the commercial paper towel have antibacterial activity following one hour of exposure to the bacterial inoculum, we co-incubated these substrates with *E. coli*. We compared the antibacterial activities of Orange PeelTowels, Lime PeelTowels, Kiwi PeelTowels, orange peels, lime peels, kiwi peels, and a commercial paper towel. We added a fixed inoculum of bacteria (10 µL; 773,000 CFU) to a small amount of each substrate in a microfuge tube. We incubated the tubes for one hour at room temperature and then applied a portion of the contents to agar plates. We incubated the plates overnight and counted colonies the next day. In nearly all cases, colonies of a single morphology were observed, suggesting that contamination had not occurred (**Figure 2**). Differing numbers of bacteria were recovered from each substrate (**Table 2**, **Figure 3A**). Of the PeelTowels, the Lime PeelTowel had the highest antimicrobial activity and reduced the bacterial inoculum by around 91%. The lime peels also killed almost all of the bacterial inoculum. Furthermore, the Orange PeelTowel and Kiwi PeelTowel both reduced the bacterial inoculums by 69% and 81%, respectively. All three PeelTowels and the lime peels killed a greater number of bacteria than the paper towel. In contrast, the paper towel, orange peels, and kiwi peels allowed the number of bacteria to increase above that present in the inoculum. While incubation

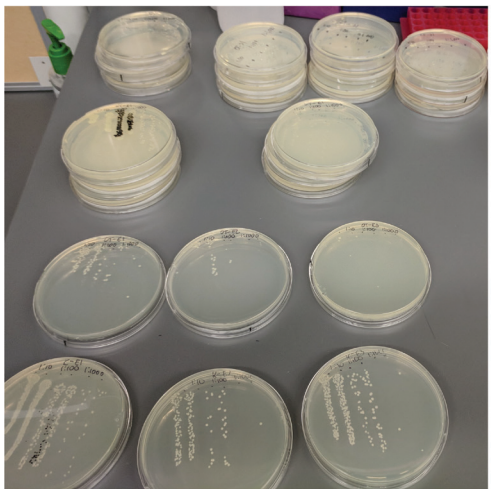

Figure 2: *E. coli* colonies growing on Lysogeny Broth (LB) agar plates for enumeration. Aliquots of 10 µL of diluted samples were spotted onto plates, which were then tilted to allow the suspension to flow across the surface of the agar. The plates were then incubated overnight at 37°C, and colonies of *E. coli* were counted.

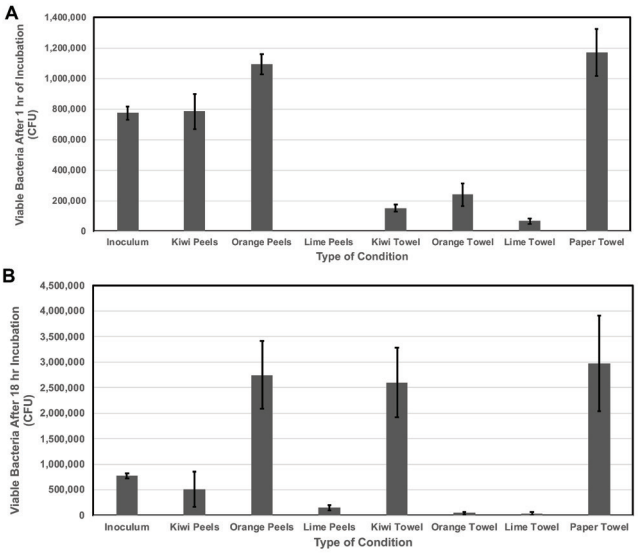

Figure 3: Viable *E. coli* following exposure to fruit peels, PeelTowels, and paper towels. *E. coli* bacteria were incubated with the indicated fruit peel, PeelTowel, or paper towel for **(A)** 1 hr or **(B)** 18 hr, and surviving bacteria were enumerated by plating. Each value represents the mean of three experiments, and each experiment represents the average CFU from two 10-µL samples. Error bars represent standard errors of the mean. When corrected for multiple comparisons, differences between the groups were not statistically significant (pairwise Mann-Whitney U tests adjusted for multiple comparisons using the Holm method, p-value > 0.05).

with the paper towel for one hour resulted in a 51% increase in bacteria, incubation with orange peels and kiwi peels yielded 41% and 2% more bacteria, respectively. Of note, none of these differences were statistically significant due to the large number of comparisons made. These results suggest that the PeelTowels have antimicrobial activity after one hour of incubation.

Some PeelTowels suppress bacterial numbers following 18 hours of exposure

We next examined whether PeelTowels, fruit peels, or the paper towel had antibacterial activity following longer incubation times with bacteria. *E. coli* bacteria (10 µL inoculum containing 773,000 CFU) were incubated with the peels and towels for 18 hours, after which viable CFU were measured by plating (**Table 2**, **Figure 3B**). The Lime PeelTowel had the greatest antimicrobial activity, killing 95% of the inoculum. The lime peels killed around 81% of the inoculum. Furthermore, both the Lime PeelTowel and Orange PeelTowel caused a decrease in the numbers of viable bacteria at 18 hours compared to the numbers in the inoculums and the paper towel. In contrast, the orange peels, paper towel, and Kiwi PeelTowel all contained more bacteria than the initial inoculum. The orange peels contained 255% more bacteria than were present in the inoculum. Likewise, the paper towel contained 286% more bacteria than were present in the inoculum -- a greater increase than was observed after one

	Number of viable bacteria after 1 hour of exposure to peels and towels		Number of viable bacteria after 18 hours of exposure to peels and towels	
PeelTowel	Viable bacteria relative to inoculum (%)	Viable bacteria relative to paper towel (%)	Viable bacteria relative to inoculum (%)	Viable bacteria relative to Paper Towel (%)
Inoculum	N/A	-34%	N/A	-74%
Kiwi peels	+2%	-33%	-34%	-83%
Orange peels	+41%	-7%	+255%	-8%
Lime peels	-100%	-100%	-81%	-95%
Kiwi towel	-81%	-87%	+236%	-13%
Orange towel	-69%	-79%	-94%	-98%
Lime towel	-91%	-94%	-95%	-99%
Paper towel	+51%	N/A	+286%	N/A

Table 2: Viable bacteria after 1 hour and 18 hours of exposure to peels and towels. The CFU of *E. coli* recovered by plating following 1 hr or 18 hr of incubation with peels or PeelTowels were compared to the starting inoculum and to the CFU recovered from paper towels incubated with bacteria for the same amount of time. N/A = not applicable. Differences between each pair of tested conditions was not statistically significant (pairwise Mann-Whitney U tests adjusted for multiple comparisons using the Holm method, p-value > 0.05).

hour of exposure. While the Kiwi PeelTowel demonstrated a reduction in the number of bacteria after one hour of exposure, after 18 hours these towels contained 236% more bacteria than were present in the inoculum. While differences in antimicrobial activity between some of the PeelTowels and fruit peels were evident, they were not statistically significant when corrected for multiple comparisons due to the large number of comparisons made.

Lime PeelTowels demonstrate significant antibacterial activity in repeat experiments

As mentioned, the large number of comparisons made prevented us from detecting statistically significant differences between the PeelTowels, fruit peels, and the paper towel. We therefore repeated the antibacterial assays using only the Lime PeelTowel, which had the highest antibacterial activity, and the paper towel. An additional change was that the Lime PeelTowel was exposed to UV irradiation prior to the assay to minimize the number of microbes on its surface prior to commencing the experiment. In this repeat assay, a new *E. coli* inoculum was generated as described above. Plating and enumeration of colonies indicated that a 10 µL volume of this inoculum contained 357,500 CFU of *E. coli*. The bacterial inoculum was incubated with the Lime PeelTowel or a paper towel for 1 hour and 18 hours. The Lime PeelTowel reduced the number of viable *E. coli* by 50% (from 357,500 CFU to 179,944 CFU) after one hour of incubation and contained 61% fewer viable bacteria than the paper towel (**Figure 4A**). The Lime PeelTowel reduced the number of viable *E. coli* from 357,500 CFU to 8,500 CFU (98%) after 18 hours of incubation (**Figure 4B**). In contrast, the paper towel contained 8,000,000 CFU of viable *E. coli*. All of these differences were statistically significant (pairwise Mann-Whitney U tests adjusted for multiple comparisons using the Holm method, *p*-value < 0.05), demonstrating the antibacterial activity of the Lime PeelTowel after 1 and 18 hours of incubation.

PeelTowels are highly absorbent

We next compared the absorption of PeelTowels and fruit peels to a paper towel. The volume of water absorbed by a 2 cm x 1 cm portion of each PeelTowel and the paper towel following immersion in water for 10 seconds was measured (**Figure 5**). The Lime PeelTowel absorbed almost three times the amount of water (0.93 mL) as the standard paper towel (0.33 mL). The Orange PeelTowel absorbed about the same amount of water as the paper towel (0.30 mL), as did the Kiwi PeelTowel (0.23 mL). In summary, the absorbency of the Lime PeelTowel was greater than that of the paper towel, Kiwi PeelTowel and Orange PeelTowel.

DISCUSSION

Today, lack of hygiene is a significant problem worldwide. Reports suggest that 35% of the world's population (2.5 billion people) in 2012 were without proper hygiene (1). Thus, there is a dire need for innovative and sustainable approaches that facilitate hand hygiene. Of note, commonly eaten fruits contain antibacterial factors that create an inhospitable environment for bacteria (6). Therefore, we designed PeelTowels made from orange, kiwi, and lime peels. Since PeelTowels may remove bacteria from hands either through antibacterial activity or by absorbing bacteria-laden water, we tested

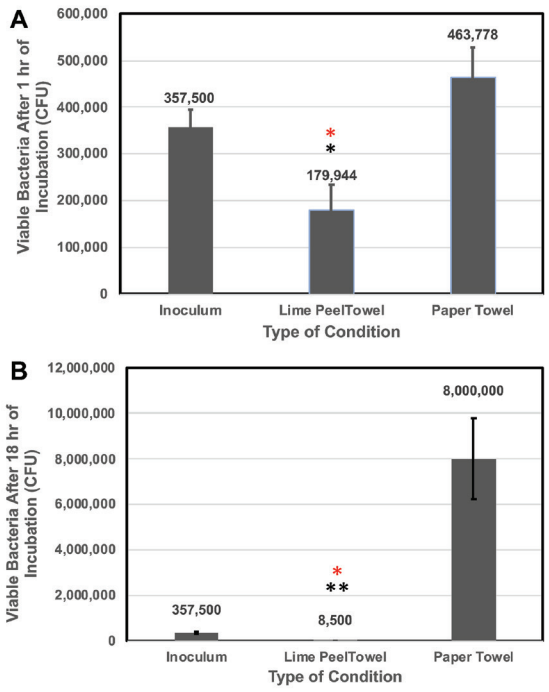

Figure 4: Viable *E. coli* following exposure to UV-irradiated Lime PeelTowels and paper towels. *E. coli* bacteria were incubated with UV-irradiated Lime PeelTowels or paper towels for **(A)** 1 hour or **(B)** 18 hours, and surviving bacteria were enumerated by plating. Each value represents the mean of three experiments, and each experiment represents the average CFU from two 10-µL samples. Error bars represent standard errors of the mean. * $p \leq 0.05$, ** $p \leq 0.01$, red asterisk is comparison to paper towel, black asterisk is comparison to inoculum (two sample independent one-tailed *t*-test, $p < 0.05$).

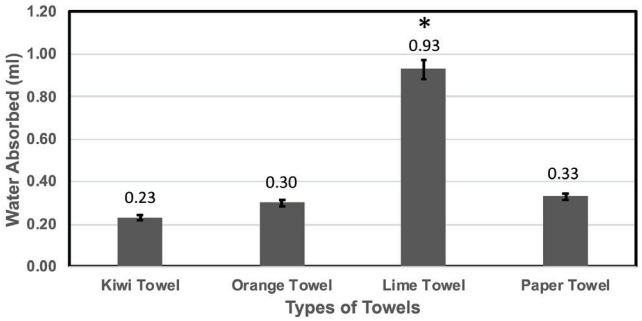

Figure 5: Water absorption by PeelTowels, fruit peels, and paper towels. The PeelTowels, fruit peels, and paper towels were immersed in water to measure their absorption. Each value represents the mean of three experiments. Error bars represent standard errors of the mean. * $p \leq 0.0005$ compared to each of the other conditions (one-way ANOVA, followed by pairwise comparisons using Tukey's multiple comparisons test).

their capacity to effectively kill bacteria and to absorb water. A potential advantage of PeelTowels is that they are eco-friendly in that they are both biodegradable and generated from waste products.

Two of the three PeelTowels were antibacterial and performed as well as or better than the paper towel. The Lime PeelTowel reduced the growth of the *E. coli* bacteria by 50-91% after 1 hour and 95-98% after 18 hours of incubation. The lime peels by themselves reduced bacterial CFU by 100% at 1 hour and 81% at 18 hours, explaining the superior performance of the Lime PeelTowels. This is consistent with reports that limes contain more citric acid than oranges or kiwi (**Table 1**). The Lime PeelTowel also absorbed about three times more water than paper towels and the Orange and Kiwi PeelTowels. This may reflect differences in the conformations and degree of drying of cellulose in lime peels compared to other peels, as both these factors may have dramatic effects on water retention by cellulose (19). The Orange PeelTowel reduced growth of the *E. coli* bacteria by 68% and 94% after 1 and 18 hours, respectively, although these differences were not statistically significant. Additionally, the Orange PeelTowel absorbed water to the same degree as the paper towel. In contrast, Kiwi PeelTowels performed well at 1 hour but poorly after 18 hours. This may be due to the presence of antimicrobial factors that are unstable and lose activity over 18 hours. For example, kiwi peels are rich in polyphenols, which have antimicrobial activity but degrade in the presence of oxygen (20-21). The short-lived activity of the Kiwi PeelTowels against *E. coli* suggests that they may not be as effective in facilitating hand hygiene as the other types of PeelTowels. Somewhat surprisingly, kiwi and orange peels exhibited no antibacterial activity after one hour of exposure, whereas Kiwi PeelTowels and Orange PeelTowels demonstrated high activity at this time point. We speculate that the additional processing (i.e. time in blender) of these peels during generation of the PeelTowels may have released more of their antibacterial compounds. These findings demonstrate that peels and PeelTowels differ substantially in their ability to kill or inhibit the growth of *E. coli*.

Our study has several limitations. Ideally, the PeelTowels would be sterile at the start of each experiment. Because our PeelTowels were homemade, contaminating bacteria or fungi were likely present at the start of our initial experiments, and these microbes may have been counted as *E. coli* CFU. However, the colonies we observed on our growth plates were of uniform color and morphology (**Figure 2**), making this possibility less likely. In addition, we repeated antibacterial experiments with UV-irradiated Lime PeelTowels and obtained similar results. A second source of error is that some bacterial colonies were not distinct from one another on the agar plates used for counting, forcing us to use our best judgement in determining whether one colony or two were present. To minimize this error, we plated several dilutions. We only tested *E. coli* bacteria, so we do not know how PeelTowels will perform against other bacteria important for hand hygiene, such as *Salmonella* and *Shigella* species. We normalized measurements of absorption to towel area rather than weight. Since the PeelTowels were thicker than the paper towels, this method of normalization favors the PeelTowels. We chose to normalize based on area because we felt individuals would use a single towel sheet (regardless of its thickness) for hand hygiene. Finally, we measured the performance of PeelTowels under laboratory conditions, which may not accurately reflect their antibacterial activities and absorption under real-use conditions.

In future studies, several additional aspects of PeelTowels need to be examined. The antibacterial effectiveness of the PeelTowels over a shorter period of time (e.g. one minute) that more realistically simulates the usage of the PeelTowel for hand hygiene should be tested. Although the PeelTowels would likely kill a smaller number of bacteria over this short time frame, it is likely that human hands would also carry fewer bacteria than the high numbers used in the current experiments. The shelf life of PeelTowels will need to be tested, since organic material is prone to degrade over time. The tensile strength of PeelTowels will also need to be examined to ensure they are capable of withstanding their intended uses. Other fruit peels with high concentrations of vitamin C, citric acid, or additional antibacterial compounds such as acetic acid, acetone, and alkaloids should also be tested (22).

In summary, we have developed a prototype of an eco-friendly paper towel substitute, which we have designated the PeelTowel, from fruit peels and paper. Lime PeelTowels effectively killed *E. coli* bacteria and successfully absorbed water. With further optimization, these PeelTowels have the potential to reduce waste while improving hand hygiene.

MATERIALS AND METHODS

Production of PeelTowels

Orange, Lime, and Kiwi PeelTowels were produced using a 5-step process. First, a molding was made from two picture frames. We cut out a piece of window screen to match the frame size and hot-glued the window screen to the back of the first frame. The other frame was lightly placed on top of the window screen to complete the molding. Second, we laid a dry cloth on a table in well-lit area and shredded 2 sheets of 8.5-inch x 11-inch paper per towel onto the cloth. Third, we washed and peeled four limes, oranges, and kiwis. For the limes, we used a lime squeezer to squeeze out the juice and retain the peel of the lime. Then, all the peels were separately ground (60-90 seconds) along with the shredded paper and 600 mL of water using a blender and made into a smooth paste. Fourth, we placed the constructed molding inside a large, square-shaped basin that was ¼ filled with water. Using the water as an aid, we spread the paste evenly on top of the window screen of the molding. Fifth, after removing the molding from the bucket, the PeelTowel was allowed to dry. After five minutes, we carefully lifted the PeelTowel from the window screen and used a sponge and hairdryer to

remove any remaining water. Dried PeelTowels were stored at room temperature. For some experiments, each side of the PeelTowel was exposed to UV irradiation for 2.5 hours in a laminar flow hood immediately prior to use. Commercially available Bounty brand paper towels (The Proctor and Gamble Company) were purchased for use as a control.

Estimation of the bacterial inoculum

Antibacterial testing was performed using aseptic technique. *E. coli* strain S17.1 (23) was grown overnight on LB agar plates at a temperature of 37ºC. An individual colony was removed from the plate technique and added to 1 mL of LB medium, which was vortexed. The OD_{600} of the suspension was measured using a spectrophotometer (BioPhotometer D30, Eppendorf, Hamburg, Germany). The OD_{600} of the bacterial suspension was then adjusted to obtain approximately 10^8 CFU/mL using published OD_{600} vs. CFU/mL curves (24). This final suspension was then used in experiments. The actual bacterial inoculum was measured by plating a portion of the suspension onto LB agar plates and counting colonies the following day, as described below.

Measurement of antibacterial activity

Approximately 1 cm x 1 cm sections of PeelTowels or paper towels were placed in microfuge tubes. Ten µL of the *E. coli* inoculum was added to the substrate in each tube. The tubes were then capped and incubated at 37°C for 1 or 18 hours. After the incubation, approximately five small sterile glass beads and 1 mL of LB medium were added to each tube, which was vortexed for approximately one minute. The number of viable bacteria in each tube was then measured by plating and counting colonies, as described below. A similar approach was used to measure the antibacterial activity of fruit peels except that peels from fruit were ground in a blender with approximately 50-150 mL of water for 30-45 seconds, and 100 µL of the resulting fruit peel purée were added to the microfuge tubes in place of the PeelTowels. Following incubation at 37°C for 1 or 18 hours, 900 µL of LB medium was added to each tube. The tube was then vortexed, serially diluted, and plated.

Estimation of bacterial numbers by plating

The number of viable bacteria in a test suspension was determined by serial dilution and plating. Briefly, we removed 100 µL from the test sample and added it to 900 µL of LB medium (1:10 dilution). The mixture was vortexed, and 100 µL was removed from it and added to 900 µL of LB (1:10^2 dilution). This process was repeated up to five times. At this point, 10 µL aliquots of the dilutions were spotted onto an agar plate, the plate was held vertically to allow the bacterial suspensions to form a streak across the plate surface. The plates were then incubated at 37°C for approximately 24 hours, after which the colonies were counted. Each measurement was the average of counts from two different 10 µL samples. The number of dilutions was taken into account to estimate the CFU in the initial test suspension. Most measurements were performed in triplicate, although a few were done in duplicate because technical difficulties caused one of the samples to be discarded.

Measurement of water absorption

The PeelTowels and paper towel were each cut into 2 cm x 1 cm rectangle pieces, and each piece was placed into a 5 mL tube filled with 3 mL of water. After waiting for ten seconds, each piece was removed using forceps. The volume of water remaining in each tube was then measured to estimate the volume of water absorbed by the PeelTowel.

Statistical analysis

Each experiment was performed in duplicate or triplicate, and the means and standard errors were calculated. For bacterial counts, differences between multiple groups were assessed using pairwise Mann-Whitney U tests, and *p*-values were adjusted for multiple comparisons using the Holm method. Differences between two samples were assessed using two sample one-tailed independent t-tests. For water absorption, differences between groups were determined by performing one-way ANOVA, followed by pairwise comparisons using Tukey's multiple comparisons test. Statistical significance was defined as an adjusted *p*-value of ≤ 0.05.

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