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Effect of Antimicrobial Washes, Essential Oil Vapor Phase, and Antimicrobial Pullulan Coating in Reducing *Escherichia coli* O157:H7 and *Salmonella* Typhimurium on Strawberries

ABSTRACT

The aim of this study was to evaluate three different antimicrobial strategies—washes, essential oil vapor phases, and coatings—against *Escherichia coli* O157:H7 and *Salmonella* Typhimurium to improve the safety of strawberries. Results indicated that 0.5% acetic acid and acidified 0.1% sodium chlorite were effective in reducing the population of *E. coli* O157:H7 (1.6 and 2.6 log CFU/g) and *Salmonella* Typhimurium (2 and 2.8 log CFU/g), respectively. Thyme essential oil vapor at 500 µL/Lair showed greater inhibition against *E. coli* O157:H7 and *Salmonella* Typhimurium on strawberry (2 and 3 log CFU/g, respectively) than oregano oil (1.5 and 2.5 log CFU/g, respectively; $P < 0.05$). During challenge studies, the coatings containing grape seed nanoparticles and pomegranate peel nanoparticles were demonstrated to effectively inhibit ($P < 0.05$) these pathogens compared with the control coatings. Overall, a greater antimicrobial activity (>4 log CFU/g) was observed when the different systems were combined, demonstrating the possibility to use these strategies to improve the quality and safety of strawberries.

INTRODUCTION

In recent years, consumer demand for fresh, healthy, and nutritious foods has increased *(62)*, posing questions on the microbial safety of handling and packing operations *(7)*. In the United States, fresh produce is ranked as the fourth food commodity responsible for foodborne outbreaks, being involved in 1.2 million illnesses, 7,100 hospitalizations, 134 human deaths, and $1.4 billion in associated costs each year *(5, 17)*. Because pathogenic bacteria can survive a wide range of pH and temperature conditions, they represent a threat for the produce industry *(53)*. Between 2009 and 2013, several U.S. states reported outbreaks linked to fresh produce, in particular, strawberries and blueberries contaminated with *Salmonella* Newport, *Escherichia coli* O157:H7, and *E. coli* O26 *(13, 15, 37, 42)*.

Strawberry (*Fragaria* × *ananassa*) is a highly desirable fruit for taste and flavor and a good source of bioactive compounds such as vitamins, minerals, anthocyanins, polyphenols, and natural antioxidants *(28)*. The quality of strawberries can decrease rapidly due to physiological stressors, water loss, fungal decay, and high respiration rate *(39)*. Therefore,

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researching strategies to ensure the safety and enhance the quality of strawberries is important for the produce industry worldwide. Egypt is the fifth largest grower of strawberries worldwide, producing approximately 350,000 tons per year *(18)*; thus, ensuring the safety of strawberries is key in Egypt. The goal of the current Egyptian policy Vision 2030 is to improve the safety of food products consumed domestically and internationally by using novel and effective techniques *(43)*. Several inactivation strategies have been suggested and investigated to inactivate pathogen contamination on strawberries by using dips or sprays *(33)*. However, in these applications, the efficacy of the antimicrobial substances has been limited due to the uncontrolled migration and inactivation of the active compounds when interacting with food components *(8)*. Other antimicrobial agents, such as essential oils (EOs) *(49)* and/or active coating techniques *(48)*, could potentially overcome these limitations by having a controlled release of active compounds and interacting directly with the microorganisms on the food *(24, 47)*.

Hence, the aim of this research was to evaluate the effectiveness of integrating systems based on antimicrobial washes and EO vapor phases as well as grape seed (GS) and pomegranate peel (PP) pullulan (Pu) coatings to reduce and control the growth of *E. coli* O157:H7 and *Salmonella* Typhimurium on experimentally inoculated strawberries stored at refrigerated temperatures for 18 days. EOs are natural active components that have been widely used in coatings for produce, such as strawberry, tomato, and grape *(4, 9, 41)*, and in vapor phase applications for animal food products *(26, 32, 49)*. Food processing by-products (such as GS and PP) have bioactive compounds, that is, phenolics and flavonoids *(1)*, that are well known for antioxidative, anti-inflammatory, and other health benefits *(29, 56)*. These by-products have gained extra attention in recent years due to their potential to improve color, flavor, and microbial quality *(36, 45)*.

Pu is a water-soluble polysaccharide obtained from *Aureobasidium pullulans (20)* that produces colorless, odorless, tasteless, transparent films impermeable to both oxygen and oil *(64)*. Several researchers have already demonstrated the effectiveness of Pu films to control the growth of postharvest fungi on strawberries *(60)* and the use of Pu coatings to improve strawberries quality and shelf life *(21)*. Nevertheless, the application of these interventions alone presents limited efficacy, whereas the integration of such systems might offer an enhanced effect on the safety and quality of fresh fruits.

MATERIALS AND METHODS

Bacterial strains

E. coli O157:H7 (ATCC 43895, American Type Culture Collection, Manassas, VA) and *Salmonella* Typhimurium (ATCC 14028, American Type Culture Collection) were the two bacterial strains used in this study. Cultures were maintained on tryptic soy agar (TSA; Biolife, Italiana, Italy). The day of the experiment, bacteria were activated twice in tryptic soy broth (Biolife) and incubated at 37°C for 16 h. Afterward, bacterial cells were harvested and centrifugated (1,500 × g at 4 ± 1°C). The obtained pellets were washed and resuspended in 10 mL of sterile 0.1% buffered peptone water (Biolife) at a final population of ~5 log CFU/mL. Bacterial count was verified by enumeration on TSA *(19)*.

Produce

Fresh strawberries (*Fragaria ananassa* cv. Camarosa) were purchased from a local farm in Qaluobia, Egypt, for the challenge study in July 2019. All fruits were selected based on the same ripening stage (three-fourths of the surface showing red), were of uniform size, and free from visible defect and decay. Pu was supplied by the Hayashibara Company (Okayama, Japan). Glycerin, xanthan gum, chlorine (C), sodium chlorite (SC), citric acid (CA), lactic acid (LA), acetic acid (AA), and calcium chloride were purchased from El-Nasr Company (Cairo, Egypt). Food-grade thyme (*Thymus vulgaris*) and oregano (*Origanum vulgare*) oils were obtained from the Pharaonic Company (Cairo, Egypt).

Antimicrobial strategies

Different strategies were evaluated: washing solutions, antimicrobial coatings, and vapor phase antimicrobial agents. Because of the delicate nature of strawberries, the washing solutions were to simulate hospitality preparation, where the fruits need to be washed and consumed immediately. The other two strategies were to simulate treatment to be used during handling and packing operations.

Washing solutions

Six different antimicrobial washing solutions, in addition to sterile tap water, were tested for efficacy on experimentally inoculated *E. coli* O157:H7 and/or *Salmonella* Typhimurium. The washing solutions used in the present study were CA (0.5% [w/v]), LA (0.5% [w/v]), AA (0.5%, [v/v]), C (100 ppm), SC (0.1% [w/v]), and acidified SC (ASC; 0.1% [w/v]).

Antimicrobial coatings

Pu coating was prepared according to the method described by Morsyet al. *(46)*, with slight modifications. Briefly, Pu (5% [w/v]) was dissolved in distilled water at 60 ± 1°C and then glycerol (2% [w/v]) and xanthan gum (0.1% [w/v]) were added. The solution was gently mixed at room temperature for ~6 h and autoclaved at 121°C for 15 min. The Pu solution was left to cool and subsequently 1% antimicrobial solutions were added.

Lyophilized GS-nanoparticles (GS-NPs) and pomegranate peel (PP)-NPs were obtained from Al-Marwa Company (Cairo, Egypt). The GS and PP were transported to the laboratory in an ice box and then treated with 200 ppm sodium hypochlorite for 2 min, washed, dried, and ground *(3)*. Subsequently, the ground GS and PP were lyophilized under the following conditions: freezing, –40 ± 1°C; dehydration,

18 ± 1°C; and condensing, −85 ± 1°C. GS and PP were then reduced to nano size according to Khataee et al. *(35)*. Briefly, GS and PP were fine ground (model MC300, Moulinex France) to micro size (110 to 150 µm) and then crushed by a high-energy planetary ball-mill (model PM 2400, Iran) to obtain nano size. The transfer process was performed using a ball mass-to-powder mass ratio of 10:1 and rotation rate of 320 rpm for 2 h under atmospheric conditions. GS-NPs and PP-NPs were measured by a Zetasizer Nano ZS instrument (NanoSight NS300, Malvern Panalytical, Malvern, UK), exhibiting with an average size of 65 ± 2 and 80 ± 2 nm, respectively. Both NPs were packed in dark glass bottles and stored until the experiments.

Antimicrobial activity of antimicrobial strategies (in vitro)

Antimicrobial activity of GS-NPs and PP-NPs was estimated using a plate overlay assay *(55)*. In brief, plates were overlaid with 10 mL of semisoft TSA (0.5% [w/v] agar) seeded with 100 µL of an overnight broth culture of *E. coli* O157:H7 and *Salmonella* Typhimurium (~5 log CFU/mL). An aliquot of 20 µL of each GS-NPs or PP-NPs at different concentrations (0.1, 0.2, 0.3, 0.5, 1, and 1.5% [w/v]) was spotted onto seeded lawns and dried in the laminar air hood for 5 min.

Pu coatings incorporated with GS-NPs or PP-NPs were cut into pieces (1 by 1 cm). The antimicrobial activity was evaluated using plate overlay assays *(44)*. Plates were scored for inhibition zones after 48 h of incubation at 37°C.

The antimicrobial activity of the EO vapor phase was determined using the inverted petri dish method *(66)*. TSA plates were inoculated with 100 µL of *E. coli* O157:H7 and *Salmonella* Typhimurium suspension (~5 log CFU/mL) and allowed to dry 5 min. Sterile filter paper disks (Whatman No. 1, Whatman, Maidstone, UK) were positioned on the center of the lid of the petri dish, and 0, 10, 50, 100, 250, and 500 µL of thyme or oregano oil was poured. The plates were tightly sealed with Parafilm to avoid vapor fugues and were incubated at 37°C for 48 h. Blanks (controls) were performed by adding 10 µL of ethanol to the filter paper disks, which was shown to have no effect on the viability of the *E. coli* O157:H7 and *Salmonella* Typhimurium. Inhibition zones were measured with a Vernier caliper, and the concentration of EOs was expressed as microliters per liter of air (µL/L_{air}).

Strawberries challenge study (in vivo)

Before each experiment, strawberries were surface treated with UV under a laminar flow hood for 15 min to reduce background microflora before artificial contamination *(52)*. Subsequently, the strawberries were spot inoculated with overnight and diluted cultures of *E. coli* O157:H7 and *Salmonella* Typhimurium at ~5 log CFU/cm^2 and dried for 20 min to allow for cell attachment. Strawberries not inoculated were used as controls.

Washing solutions

Inoculated strawberries were washed with 0.5% CA, 0.5% LA, 0.5% AA, 100 ppm C, 0.1% SC, and 0.1% ASC by immersion and spray for 2 min *(30)*. The spray method was performed using a multifunction manual pressure sprayer 2L (Ningbo Synkemi Co., Ltd., Zhejiang, China) with a flow rate of 6.5 mL/s. The nozzle was placed vertical at 20 cm over the fruits twice each side for 2 min *(30)*.

EO vapor phases

Strawberries were placed in 500-mL hermetic seal transparent glass jars (Middel East of Glass Co., Cairo, Egypt). Sterile filter paper disks (Whatman No. 1) were taped on the container, and thyme or oregano oil at 500 µL/L_{air} (EO volumes were adjusted to the total volume of the container) was poured on disks. The strawberries were placed on the containers' lids, and after pouring the EOs, the containers were closed (upside down) and sealed with Parafilm to avoid vapor fugues. A blank sample (control) was prepared by adding 10 µL of ethanol to the filter paper discs, which was revealed to have no impact on the viability of the *E. coli* O157:H7 and *Salmonella* Typhimurium. The jars were kept at 4 ± 1°C for 18 days *(67)*. The concentration of thyme or oregano EO was expressed as microliters per liter of air. The experiment was performed in triplicates. The jars were kept at 4 ± 1°C for 18 days *(66)*.

Pu coatings

Strawberries were coated with pullulan solution (5% [w/v]) one by one and then allowed to dry aseptically in a biological safety cabinet at ambient temperature. Several treatments were performed: uncoated (control), water wax with thiabendazole as positive control *(67)*, pullulan (Pu), Pu with GS-NPs (Pu-GS-NPs), Pu with thyme oil vapor and GS-NPs (Pu-TOV-GS-NPs), Pu with PP-NPs (Pu-PP-NPs), and Pu with TOV and PP-NPs (Pu-TOV-PP-NPs) *(58)*. The coated strawberries were placed in a container and kept at 4 ± 1°C for 18 days.

Microbiological assay

On days 0, 3, 6, 9, 12, 15, and 18, 10 ± 1 g of strawberries was aseptically transferred into 90 mL of 0.1% buffered peptone water (Himedia, Rabat, Morocco) and stomached for 2 min (model G560E mixer, Scientific Industries, Inc., Bohemia, NY). Next, serial dilutions (10-fold) were made. An aliquot of 100 µL of each dilution was spread plated in duplicates onto sorbitol MacConkey agar (Himedia) for *E. coli* O157:H7 and xylose lysine desoxycholate agar (Himedia) for *Salmonella* Typhimurium. All plates were incubated at 37°C for 24 to 48 h, and the remaining colonies were counted and expressed as log CFU per gram *(44)*.

Statistical analysis

Data from the challenge study were statistically analyzed for remaining bacteria by using one-way analysis of variance

with a significance level of $P \leq 0.05$ (SPSS 20, IBM, Armonk, NY). Data were analyzed based on a completely randomized design *(57)*. The challenge experiments were conducted in triplicate for each treatment ($n = 3$). Multiple mean comparisons were carried out applying least significant difference and Duncan's test *(50)*.

RESULTS AND DISCUSSION

Foodborne pathogens in fresh strawberries

In a preliminary study conducted at the Department of Food Technology, Benha University (Qaluobia, Egypt), during July 2019, 20 samples of strawberries (2.5 kg for each sample) were investigated for the presence of *E. coli* O157:H7, *Listeria monocytogenes*, *Salmonella* Typhimurium, and other foodborne pathogens. The results demonstrated that 85% of samples were positive for *E. coli* O157:H7 and 75% for *Salmonella* Typhimurium *(Table 1)*. Based on these observations and on *Salmonella* Newport, *E. coli* O157:H7, and *E. coli* O26 being linked to contaminated strawberries and blueberries in the United States between 2009 and 2013, these pathogens were selected in the present study *(13, 15, 37, 42)*. Furthermore, because this research represents an initial framework to understand the feasibility of several intervention strategies for produce safety, only a single strain of *E. coli* and *Salmonella* were used as model strains.

Washing solutions and reduction of foodborne pathogens on strawberries

The effect of different washing solutions (immersion or spray) was evaluated and is reported in *Figures 1 and 2*. Washing strawberries with tap water for 2 min did not significantly reduce ($P > 0.05$) *Salmonella* Typhimurium and *E. coli* O157:H7. Among organic acids, after AA, a significant reduction ($P < 0.05$) of 1.60 and 2 log CFU/g was observed

TABLE 1. Survey of selected foodborne pathogens in fresh strawberry conducted in Egypt during summer 2019. The symbol (+) indicates that the microorganism was detected, while the symbol (-) indicates that the microorganism was not detected

Sample ID	Microorganisms			
	E. coli O157:H7	*L. monocytogenes*	*Salmonella* Typhimurium	*S. aureus*
1	+	–	+	–
2	+	–	–	+
3	–	–	+	–
4	+	–	+	–
5	+	–	+	–
6	+	–	+	–
7	–	–	+	–
8	+	–	+	–
9	+	–	+	–
10	–	–	+	–
11	+	–	–	–
12	+	–	+	–
13	+	–	+	–
14	–	–	+	+
15	+	–	+	–
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20	+	–	+	–

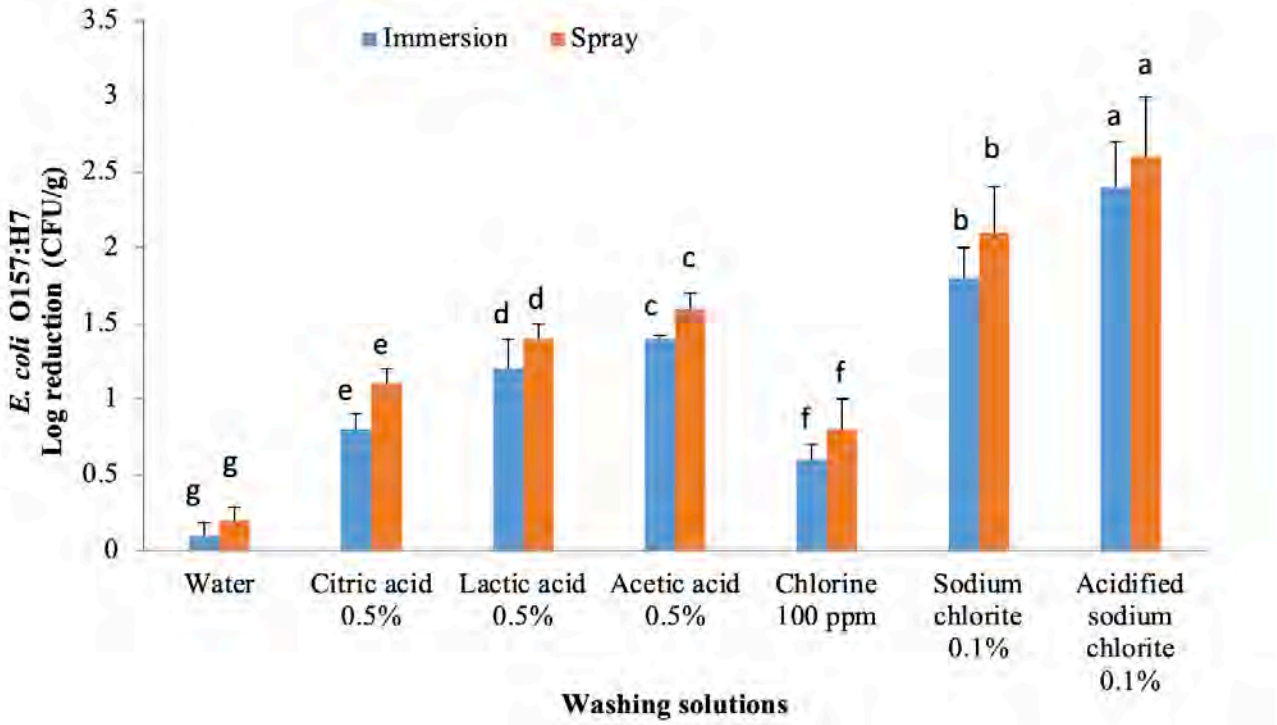

FIGURE 1. Effect of antimicrobial washes on experimentally inoculated strawberries with *E. coli* O157:H7, reported as log reduction (CFU/g). Values are the average of triplicate samples from each of three experiments ($n = 9$); error bars represent SD.

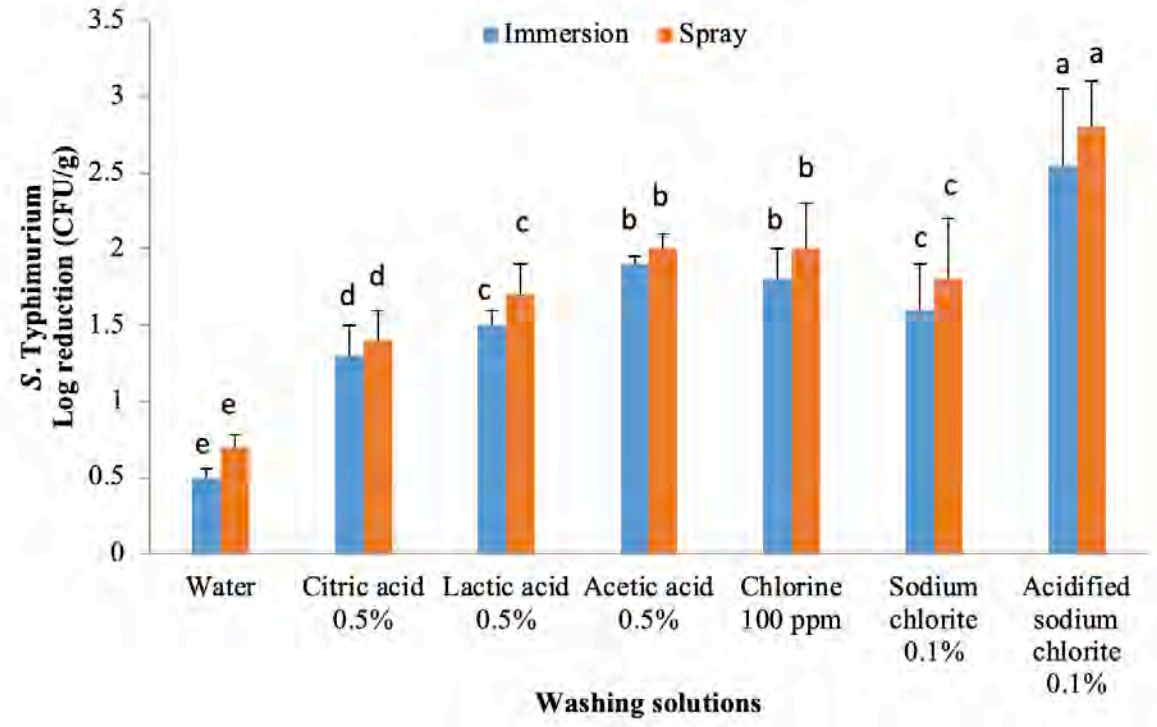

FIGURE 2. Effect of antimicrobial washes on experimentally inoculated strawberries with *Salmonella* Typhimurium, reported as log reduction (CFU/g). Values are the average of triplicate samples from each of three experiments ($n = 9$); error bars represent SD.

for *E. coli* O157:H7 and *Salmonella* Typhimurium, respectively, compared with CA (1.10 and 1.40 log CFU/g) and LA (1.40 and 1.70 log CFU/g). Previous studies demonstrated that treatments with LA reduced *E. coli* O157:H7 (1.74 log CFU/g) and *Salmonella* Typhimurium (1.73 log CFU/g) on fresh organic lettuce *(51)*, suggesting also that low pH, the ratio of undissociated ions, cell physiology, and metabolism influence the efficacy of organic acids against foodborne pathogens *(52)*. Overall, organic acid solutions applied by spray showed a significantly greater reduction for both *E. coli* O157:H7 and *Salmonella* Typhimurium than immersion applications ($P \leq 0.05$). Also, the use of chemical sanitizers (C, SC, and ASC) showed a significant reduction ($P \leq 0.05$) on samples inoculated with both *E. coli* O157:H7 and *Salmonella* Typhimurium in the order ASC > SC > C. The antimicrobial capacity of ASC was higher than that of SC and C at 2.6 and 2.8 log CFU/g for *E. coli* O157:H7 and *Salmonella* Typhimurium, respectively. Previous studies reported the ability of ASC to reduce microbial population on strawberries and cherry tomatoes by 2 to 3 log CFU/g *(2, 63)*.

EO vapor phase and reduction of foodborne pathogens in vitro and on strawberries

As shown in *Table 2*, the antimicrobial activity of thyme and oregano oil vapor phase at different levels (10, 50, 100, 250, and 500 µL) against *E. coli* O157:H7 and *Salmonella* Typhimurium was evaluated. Both EOs in vapor phase were active against the tested foodborne pathogens. The diameters of inhibition zone gradually increased when the level of EOs increased. Thyme and oregano oil vapor phase produced a greater inhibition halo against *Salmonella* Typhimurium than *E. coli* O157:H7. Conversely, thyme oil was more effective than oregano oil overall. The mode of action of the EOs against bacteria is due to cell membrane damage, increased permeability, and phosphate ion leakage *(10, 38)*.

The antimicrobial activity of EOs vapor phase against *Salmonella* Typhimurium and *E. coli* O157:H7 on refrigerated strawberries is reported in *Figures 3 and 4*. Bacterial populations remained constant during the 18 days of refrigeration in the control sample. Conversely, samples exposed to EOs vapor phase showed a 1-log reduction in *Salmonella* Typhimurium populations after 3 day, and a significant reduction was observed for *E. coli* O157:H7 compared with the control sample. During storage, EOs vapor phase showed reduction of the bacterial populations (3 to 4 log CFU/g) on strawberries up to 18 days. Previous studies have found that EOs vapor phase has a greater antimicrobial impact than liquid EOs in direct contact *(31, 49, 60)*. Other researchers *(47)* demonstrated instead that lipophilic molecules in the aqueous solutions (micelles) restrain the activity of EOs against microorganisms.

Pullulan coating incorporation in GS-NPs and PP-NPs for improving quality and safety of strawberries

Antimicrobial activity of NPs by using plate overlay assays

The influence of NPs from GS and PP were evaluated and reported in *Table 3*. Both GS-NPs and PP-NPs were active at

TABLE 2. Antibacterial activity of thyme and oregano oil vapor phase against *E. coli* and *Salmonella* Typhimurium in vitro reported as inhibition halos

Treatment (µL), $n = 9$	Oil vol (µL)	Inhibition halo (cm)[a]	
		E. coli O157:H7	*Salmonella* Typhimurium
Thyme oil vapor phase	10	0.5 ± 0.05 eB	0.8 ± 0.05 eA
	50	1 ± 0.08 dB	1.2 ± 0.1 dA
	100	1.35 ± 0.13 cB	1.6 ± 0.15 cA
	250	2.15 ± 0.15 bB	2.35 ± 0.17 bA
	500	2.45 ± 0.2 aB	2.65 ± 0.2 aA
Oregano oil vapor phase	10	0.4 ± 0.05 dB	0.7 ± 0.05 fA
	50	0.8 ± 0.08 cB	1.08 ± 0.05 eA
	100	1 ± 0.1 cB	1.51 ± 0.05 dA
	250	1.7 ± 0.1 bB	2 ± 0.1 cA
	500	2.05 ± 0.15 bB	2.25 ± 0.1 bA

[a] There are no significant differences between any two means (±SD) in the same column that have the same lowercase letter ($P \geq 0.05$). There are no significant differences between any two means (±SD) in the same row that have the same uppercase letter ($P \geq 0.05$).

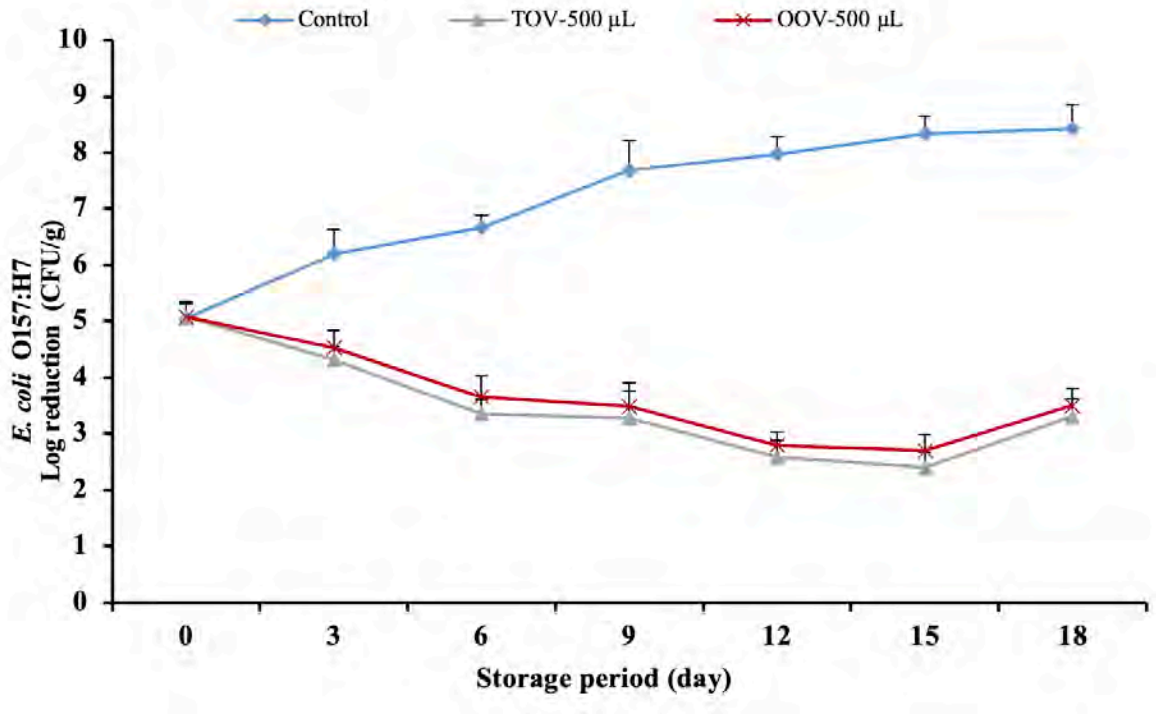

FIGURE 3. Effect of essential oils vapor phase against of *E. coli* O157:H7 on refrigerated strawberries during storage, reported as log reduction (CFU/g). Values are the average of triplicate samples from each of three experiments ($n = 9$); error bars represent SD. TOV-500 µL, thyme oil vapor (500 µL/L_{air}); OOV-500 µL, oregano oil vapor (500 µL/L_{air}).

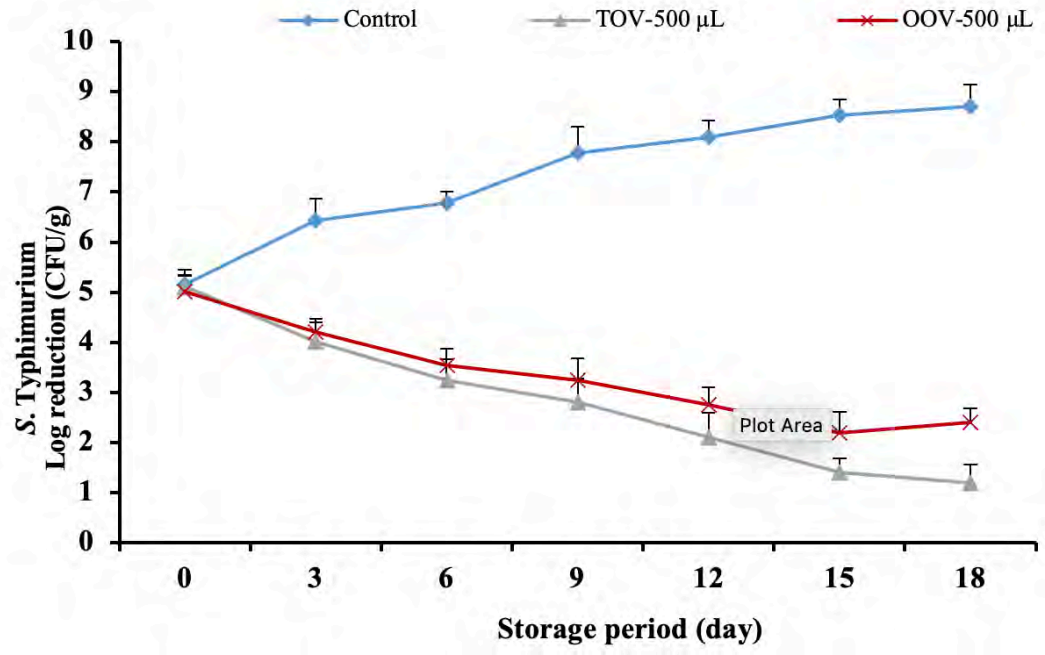

FIGURE 4. Effect of essential oils vapor phase against of *Salmonella* Typhimurium on refrigerated strawberries during storage, reported as log reduction (CFU/g). Values are the average of triplicate samples from each of three experiments ($n = 9$); error bars represent SD. TOV-500 µL, thyme oil vapor (500 µL/L_{air}); OOV-500 µL, oregano oil vapor (500 µL/L_{air}).

TABLE 3. Antibacterial activity of GS-NPs and PP-NPs against *E. coli* O157:H7 and *Salmonella* Typhimurium reported as inhibition halos

Treatment ($n = 9$)	Concn (%)	Inhibition halo (cm)[a]	
		E. coli O157:H7	*Salmonella* Typhimurium
GS-NPs	0.1	0.8 ± 0.10 eB	1.5 ± 0.07 eA
	0.2	1.4 ± 0.18 dB	1.7 ± 0.12 dA
	0.3	1.65 ± 0.22 cB	2 ± 0.26 cA
	0.5	2.37 ± 0.18 bB	2.52 ± 0.17 bA
	1	2.53 ± 0.28 aB	2.82 ± 0.22 aA
	1.5	2.65 ± 0.16 aB	2.94 ± 0.15 aA
PP-NPs	0.1	0.5 ± 0.05 dB	1.03 ± 0.05 fA
	0.2	1 ± 0.07 cB	1.28 ± 0.06 eA
	0.3	1.1 ± 0.10 cB	1.63 ± 0.07 dA
	0.5	1.83 ± 0.11 cB	2.2 ± 0.15 bA
	1	1.9 ± 0.10 bB	2.23 ± 0.12 bA
	1.5	2.2 ± 0.12 aB	2.5 ± 0.18 aA

[a] There are no significant differences between any two means (±SD) in the same column that have the same lowercase letter ($P \geq 0.05$). There are no significant differences between any two means (±SD) in the same row that have the same uppercase letter ($P \geq 0.05$).

high concentration against *E. coli* O157:H7 and *Salmonella* Typhimurium. The inhibition zone gradually increased as the concentration of NPs increased. GS-NPs and PP-NPs were more active against *Salmonella* Typhimurium than *E. coli* O157:H7, and GS-NPs demonstrated a greater antimicrobial activity overall. These results are in agreement with those of previous studies *(27, 34)* where GS extract at 0.5 and 1.5% concentrations showed a bacteriostatic effect against *E. coli* O157:H7 and *Salmonella* Typhimurium. In another study *(41)*, PP extract inhibited the growth of *Salmonella* Typhimurium.

The antimicrobial efficacy of the Pu coatings in the plate overlay assays is reported in *Table 4*. Pu coatings with 1.5% GS-NPs or PP-NPs were more effective against *Salmonella* Typhimurium and *E. coli* O157:H7. A significant difference ($P \leq 0.05$) between GS-NPs or PP-NPs against *E. coli* O157:H7 and *Salmonella* Typhimurium at different concentrations was noted. The results demonstrated no significant difference ($P \geq 0.05$) between GS-NPs at 1 and 1.5% against *E. coli* O157:H7. Similar results were observed for PP-NPs. Based on these observations, 1% GS-NPs or PP-NPs might allow better and controlled migration of the antimicrobial agents against bacteria, as reported previously *(25)*. Furthermore, in another study *(65)*, chitosan film containing PP extract exhibited antibacterial activity against *E. coli* and *Salmonella aureus*.

The antimicrobial impact of GS-NPs and PP-NPs can be attributed to several mechanisms: (i) polyphenols can penetrate the bacterial membranes and react with proteins or the cytoplasm *(12, 23)*; (ii) tannins could be able to inhibit extracellular microbial enzymes *(22, 54)*; and (iii) hydroxycinnamic acids are less polar than hydroxybenzoic acids, thereby facilitating their passage across the bacterial cell membrane.

Strawberries coating and challenge study

Based on the results obtained in the previously described experiments, TOV and Pu coating including GS-NPs and/or PP-NPs at 1% were evaluated for their combined antimicrobial efficacy on experimentally inoculated strawberries with *E. coli* O157:H7 and *Salmonella* Typhimurium at refrigerated storage (4°C) for up to 18 days. *Figure 5* shows the antimicrobial activity of Pu coating with and without the addition of the antimicrobial agents against *E. coli* O157:H7 on strawberries. Bacterial populations remained constant during storage up to 18 days in the control sample, whereas Pu-coated strawberries containing GS-NPs or PP-NPs showed a 2-log reduction in *E. coli* O157:H7 populations after 3 days. This reduction remained constant until the end of the challenge study. The samples treated with TOV and Pu coating achieved a greater reduction (~4 log CFU/g) by day 18. The combination of the antimicrobial systems was more effective than the commercial coating of fruits, that is, water wax with thiabendazole (positive control). Similar results were observed for strawberries inoculated with *Salmonella* Typhimurium *(Fig. 6)*. Our findings are in agreement with data reported by Yun et al. (66), who found that EOs vapor phase reduced more than 5 log CFU/g *Salmonella* Typhimurium on cherry tomatoes, while maintaining quality.

TABLE 4. Antibacterial activity of pullulan coating incorporation GS-NPs and PP-NPs against *E. coli* O157:H7 and *Salmonella* Typhimurium reported as inhibition halos

Treatment ($n = 9$)	Concn (%)	Inhibition halo (cm)[a]	
		E. coli O157:H7	*Salmonella* Typhimurium
GS-NPs	0.5	2.45 ± 0.15 bB	2.65 ± 0.15 bA
	1	2.7 ± 0.18 aB	2.95 ± 0.20 aA
	1.5	2.88 ± 0.15 aB	3.15 ± 0.17 aA
PP-NPs	0.5	2 ± 0.13 bB	2.3 ± 0.10 bA
	1	2.3 ± 0.15 aB	2.6 ± 0.15 aA
	1.5	2.4 ± 0.20 aB	2.7 ± 0.20 aA

[a] There are no significant differences between any two means (±SD) in the same column that have the same lowercase letter ($P \geq 0.05$). There are no significant differences between any two means (±SD) in the same row that have the same uppercase letter ($P \geq 0.05$).

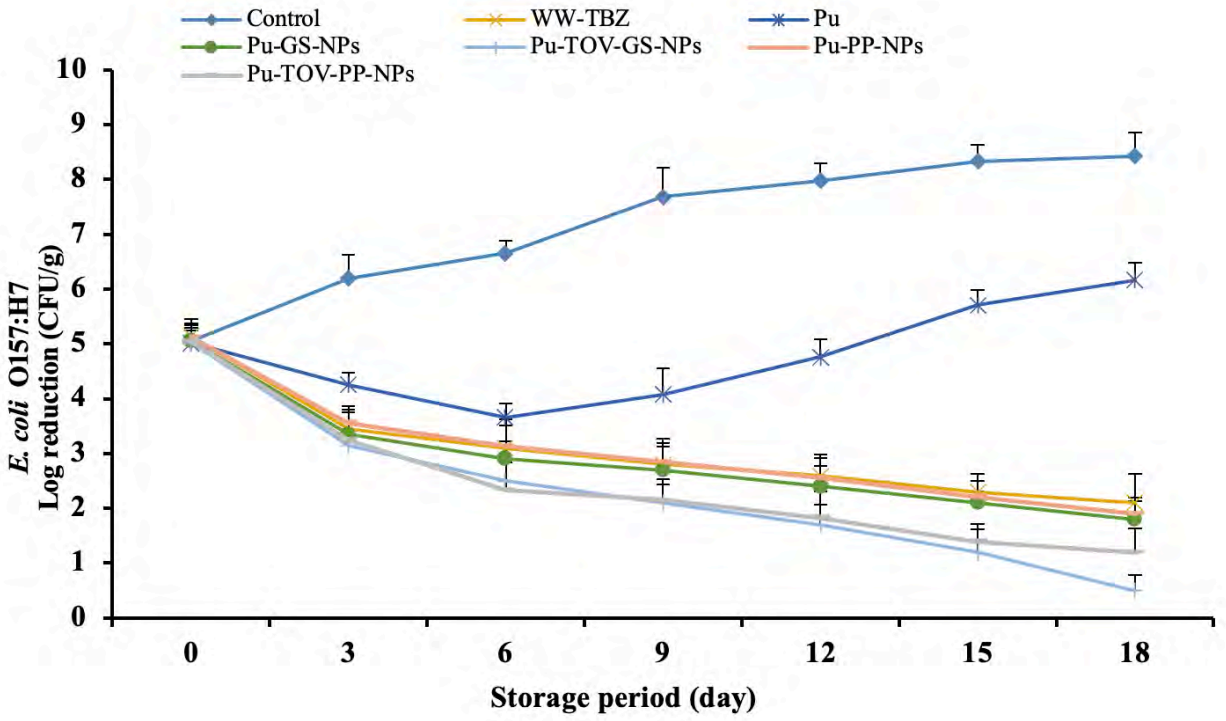

FIGURE 5. Effect of antimicrobial coating-based pullulan (Pu) incorporated with grape seed nanoparticles (GS-NPs) and pomegranate peel (PP)-NPs against of *E. coli* O157:H7 on refrigerated strawberries during storage, reported as log reduction (CFU/g). Values are the average of triplicate samples from each of three experiments ($n = 9$); error bars represent SD. OOV, oregano oil vapor; TOV, thyme oil vapor; WW-TBZ, water wax with thiabendazole.

CONCLUSIONS

Globalization of the food supply chain means new food safety risks. The produce trade has expanded and become more diverse in the variety of fruits and vegetables offered. This increased trade provides U.S. consumers with many benefits, including the possibility of improved nutrition, because these products are available year-round. Nevertheless, because of the increasing number of outbreaks linked to imported produce, retailers and buyers have started demanding high safety standards, increasing issues related to international trade to guard and enhance national food supplies. The U.S. Food and Drug Administration has been active in promoting im-

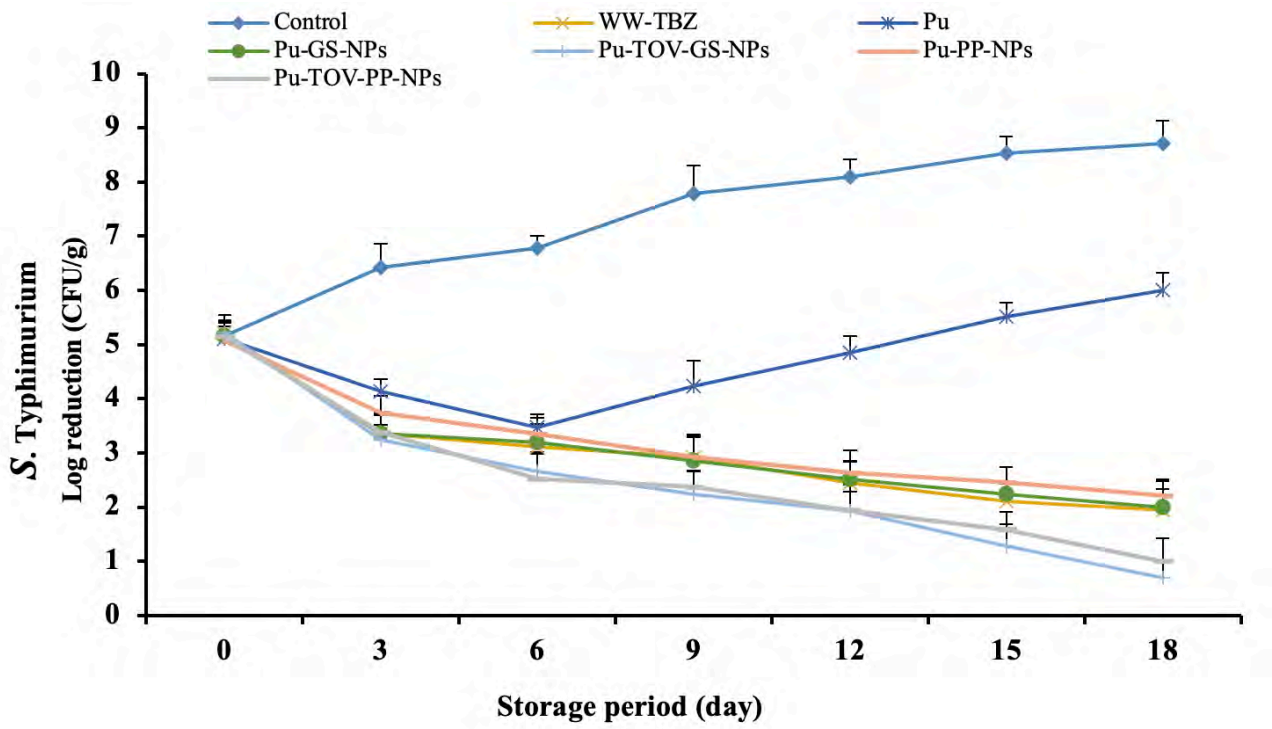

FIGURE 6. Effect of antimicrobial coating-based pulullan (Pu) incorporation grape seed nanoparticles (GS-NPs) and pomegranate peel (PP)-NPs against of *Salmonella* Typhimurium of refrigerated strawberries during storage, reported as log reduction (CFU/g). Values are the average of triplicate samples from each of three experiments ($n = 9$); error bars represent SD. OOV, oregano oil vapor; TOV, thyme oil vapor; WW-TBZ, water wax with thiabendazole.

proved food safety measures in foreign countries. This article highlights the increased focus of the Egyptian government on the food safety program to help resolve U.S. import alerts and allow secure imports of produce from Egypt to our nation, following U.S. regulations.

Results from this research demonstrated that antimicrobial washes, EOs vapor phases, and antimicrobial Pu coatings have the potential to improve the quality and safety of strawberries: antimicrobial washes, that is, 0.5% AA and 0.1% ASC, were effective against *E. coli* O157:H7 and *Salmonella* Typhimurium. TOV effectively inhibited *E. coli* O157:H7 and *Salmonella* Typhimurium on strawberries up to 18 days. NPs from GS and PP integrated into the biopolymer and applied to fresh fruits and vegetables can inhibit foodborne pathogens over an 18-day storage period at refrigerated conditions.

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Conflicts of interest

The authors declare that there are no conflicts of interest.

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