Electronic Supplementary Material (ESI) for Environmental Science: Nano.

A Novel Antimicrobial Technology to Enhance Food Safety and Quality of Leafy Vegetables using Engineered Water Nanostructures

Runze Huang[a‡$], Nachiket Vaze[a‡], Anand Soorneedi[b], Matthew D. Moore[b], Yaguang Luo[c], Elena Poverenov[d], Victor Rodov[d], Philip Demokritou[a,*]

[a] Center for Nanotechnology and Nanotoxicology, Department of Environmental Health, Harvard T. H. Chan School of Public Health, Harvard University, Boston, MA, 02115, USA

[b] Department of Food Science, University of Massachusetts Amherst, Amherst, MA, 01003, USA

[c] Environmental Microbial and Food Safety Laboratory, U. S. Department of Agriculture, Agricultural Research Service, Beltsville Agricultural Research Center, Beltsville, MD, 20705, USA

[d] Institute of Postharvest and Food Sciences, Agricultural Research Organization, The Volcani Center, Rishon LeZion, 7528809, Israel

[*]**Corresponding author**: Philp Demokritou (pdemokri@hsph.harvard.edu); 665 Huntington Avenue, Building 1, Room 1310B, Boston, MA 02115, USA

‡ These authors contributed equally $ Posthumous credit

Table of Contents

Fig. S1 Inactivation of *E. coli* on stainless steel coupons from various EWNS based nano-sanitizers synthesized with (a) single and (b) combined active ingredients. Microorganisms were inoculated on stainless steel coupon and exposed to the EWNS based nano-sanitizers synthesized with various single and combined active ingredients. The coupon was inoculated with ~10^6 CFU/coupon of *E. coli*. The coupons were then exposed to the EWNS based nano-sanitizers for 30 s as shown in Fig. 1. The temperature and relative humidity were ~ 24 °C and ~ 40%, respectively. The error bar represents 1 standard deviation. (Contains data adapted with permission from Huang, R., Vaze, N., Soorneedi, A., Moore, M. D., Xue, Y., Bello, D., & Demokritou, P. (2019). Inactivation of Hand Hygiene-Related Pathogens Using Engineered Water Nanostructures. ACS Sustainable Chemistry & Engineering, 7(24), 19761-19769. Copyright 2019, American Chemical Society).

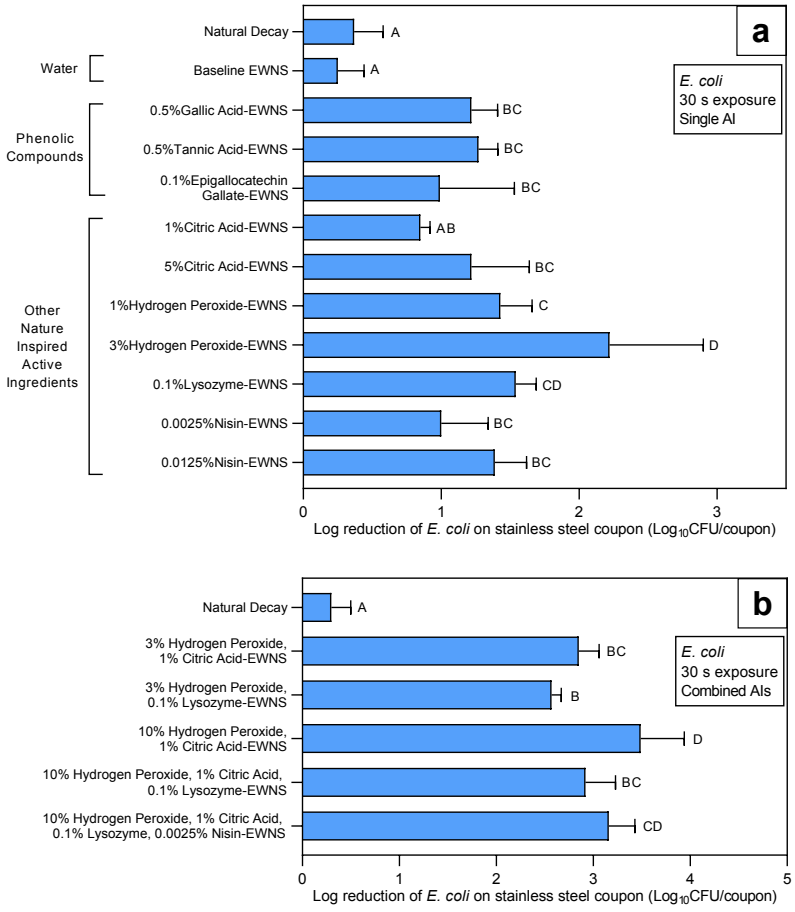

Fig. S2 Inactivation of various microorganisms on stainless steel coupon from EWNS based nano-sanitizers synthesized with an AI cocktail: (**a**): *Escherichia coli*, (**b**) *Listeria innocua*, (**c**) *Peniciilium italicum* and (**d**) *Pseudomonas fluorescens*. Microorganisms were inoculated on stainless steel coupon and exposed to the EWNS based nano-sanitizers synthesized with a combination of AIs (10% hydrogen peroxide, 1% citric acid, 0.1% lysozyme and 0.0025% nisin). The coupon was inoculated with ~10^6 CFU/coupon of *E. coli*, ~10^6 CFU/coupon of *L.* innocua, ~10^6 PFU/coupon of bacteriophage MS2, ~10^5 CFU/coupon of *P. italicum* or ~10^6 CFU/coupon of *Ps. fluorescens*. The coupons were then exposed to the EWNS based nano-sanitizers for 0 – 15 min as shown in Fig. 1. The temperature and relative humidity were ~ 24 °C and ~ 40%, respectively. The error bar represents 1 standard deviation.

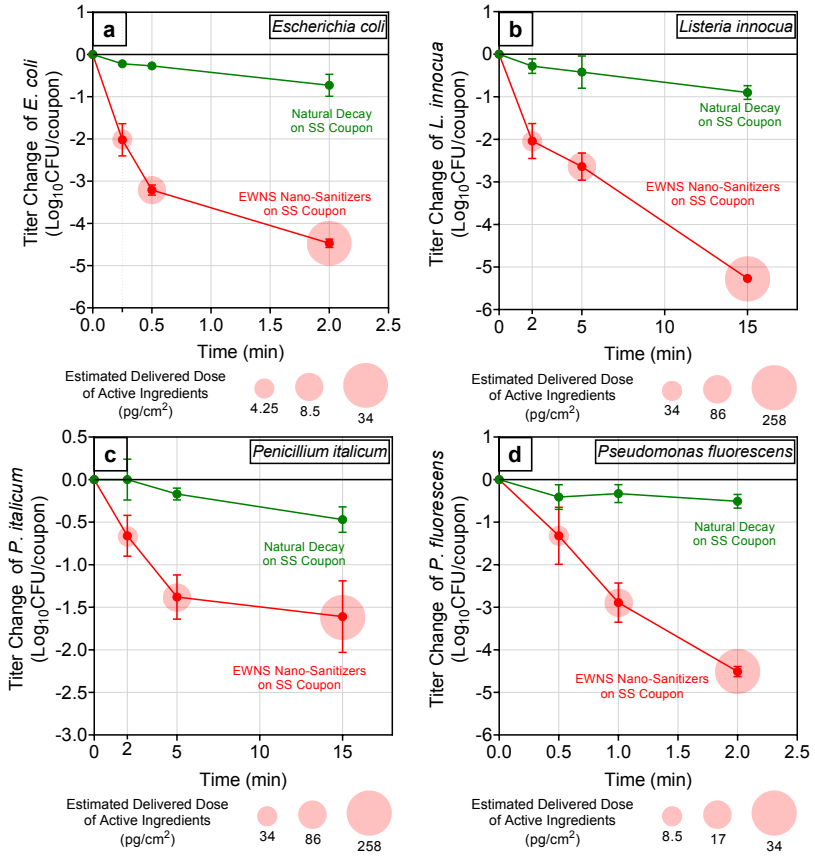

Table S1. Physio-chemical characterization of the EWNS based nano-sanitizer synthesized with 10% hydrogen peroxide, 1% citric acid, 0.1% lysozyme and 0.0025% nisin. (Adapted with permission from Huang, R., Vaze, N., Soorneedi, A., Moore, M. D., Xue, Y., Bello, D., & Demokritou, P. (2019). Inactivation of Hand Hygiene-Related Pathogens Using Engineered Water Nanostructures. ACS Sustainable Chemistry & Engineering, 7(24), 19761-19769. Copyright 2019, American Chemical Society)

Physical Characterization		
SMPS-measured mean diameter (nm)	21.0 ± 2.6	
CPC-measured concentration (number of particles/cm^3)	181090 ± 45371	
Electric charge (e^-/particle)	10.4 ± 1.6	
Chemical Characterization		
	Estimated Concentration	Measured Concentration
Short-lived ROS (mol H_2O_2 equivalent unit/particle)	N/A	$1.88 \pm 0.72 \times 10^{-14}$
Total ROS (mol H_2O_2 equivalent unit/particle)	N/A	$3.41 \pm 0.33 \times 10^{-14}$
Citric acid (ng/particle)	$4.86 \pm 1.55 \times 10^{-11}$	$< 2.47 \pm 0.41 \times 10^{-9}$ *

N/A: Not applicable.
*: 1 replicate was below the limit of detection out of a total of 3 replicates.
The detection limit of short-lived ROS and total ROS is 1.38×10^{-10} nmol H_2O_2 equivalent unit/particle.
The detection limit of citric acid is 1.84×10^{-9} ng/particle.

Details on the plaque assay utilized from Su and D'Souza:

E. coli B-15597 was grown in TSB containing 0.1% glucose, 2 mM CaCl2, and 10 μg/ml thiamine for 6 h. MS2 treated with GSE or water after neutralization with TSB containing 3% beef extract was serially diluted in TSB, and 0.7 ml of diluted phage was mixed with 0.3 ml of 6-

h E. coli host. The 1-ml host-virus combination was then added to 8 ml of 0.6% molten top agar, mixed and poured on tryptic soy agar (TSA) bottom agar plates, and incubated at 37°C overnight before counting (Su & D'Souza, 2011).

References:

Huang, R., Vaze, N., Soorneedi, A., Moore, M. D., Xue, Y., Bello, D., & Demokritou, P. (2019). Inactivation of Hand Hygiene-Related Pathogens Using Engineered Water Nanostructures. *ACS Sustainable Chemistry & Engineering*, *7*(24), 19761–19769.

Vaze, N., Pyrgiotakis, G., Mena, L., Baumann, R., Demokritou, A., Ericsson, M., … Demokritou, P. (2019). A nano-carrier platform for the targeted delivery of nature-inspired antimicrobials using Engineered Water Nanostructures for food safety applications. *Food Control*, *96*, 365–374.

Su, X., & D'Souza, D. H. (2011). Grape seed extract for control of human enteric viruses. *Applied and Environmental Microbiology*, *77*(12), 3982–3987. https://doi.org/10.1128/AEM.00193-11