Microbial Inactivation by Engineered Water Nanostructures for Enhanced Food Safety

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In the field of food safety and quality, there is a growing need to develop innovative and sustainable approaches that do not involve chemicals, in order to combat foodborne illnesses. Among the various types of food, fresh produce has garnered increased attention from regulatory authorities, producers, and consumers, demanding stricter measures to ensure safety and quality. Fresh produce has been identified as a significant source of harmful microorganisms and has been associated with serious outbreaks of foodborne diseases. As the global trend towards healthier lifestyles continues, the consumption of fresh produce is on the rise. However, existing methods for disinfecting fresh produce heavily rely on the use of chemicals or irradiation, which are both flawed and face objections from consumers. Recently a new and innovative approach has emerged for eliminating microorganisms from surfaces such as fresh produce and stainless steel, as well as from the air. This method utilizes nanotechnology and does not require the use of chemicals. It involves the creation of engineered water nano-structures (EWNS) through the process of condensation, electrospraying, and ionization of water vapor present in the atmosphere. (Pyrgiotakis *et al*., 2015).

Engineered water nano-structures (EWNS):

EWNS are nanosized water droplets carrying a strong electric charge and filled with free radicals, are produced by transforming atmospheric water vapor through electrospray. These engineered water nano-structures exhibit unique physical, chemical, morphological, and biological characteristics. With an average size of 25 nm, they contain reactive oxygen species (ROS) such as hydroxyl and superoxide radicals. Additionally, EWNS possess a high electrical charge of around 10 electrons per particle on average. They are highly mobile and have an extended lifespan. When applied to food surfaces, EWNS have the ability to deactivate microorganisms responsible for foodborne diseases, making them a promising environmentally friendly method for disinfecting food (Pyrgiotakis *et al*., 2015; Pyrgiotakis *et al*., 2014).

Synthesis of EWNS:

A single "needle" EWNS generation system was developed to understand the EWNS synthesis processes. The process involves condensing atmospheric water vapor onto a Peltier element, which is then directed through tubing to a stainless steel needle (metal capillary). The metal needle is connected to a high voltage source and positioned above a grounded electrode, with the distance between them adjustable. By applying a high voltage (-6.5 kV) between the metal capillary and the counter electrode, two distinct phenomena occur: electrospraying and water ionization **(Fig.1)**

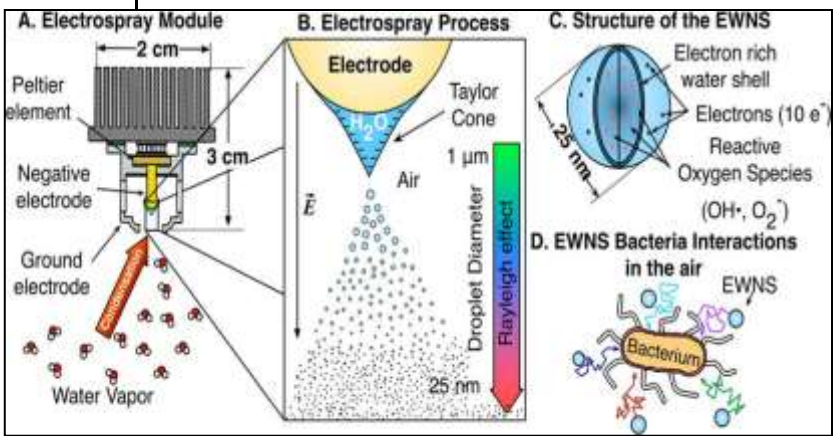

Fig.1. Synthesis of EWNS

Under the influence of the strong electric field between the electrodes, negative charges accumulate on the surface of the condensed water at the tip of the needle, resulting in the formation of a Taylor cone. This leads to the production of highly charged water droplets that continue to break into smaller particles according to the Rayleigh theory. Simultaneously, the high electric field causes some water molecules to undergo ionization, where electrons are stripped off. This process generates a significant number of reactive oxygen species (ROS). The ROS produced are encapsulated within the forming water droplets, resulting in the formation of EWNS. These engineered particles have a diameter of only 25 nanometers, making them 4,000 times smaller than the width of a human hair. Due to their high electric charge, they can remain suspended in the air for hours under indoor conditions. The small size of these nanoparticles enables them to be highly mobile, acting like tiny bombs that can move around and interact with microorganisms. By delivering the encapsulated ROS payload, EWNS can effectively deactivate microorganisms they come into contact with. Two different ways of delivering EWNS on surfaces were developed. In one method, EWNS were delivered to surfaces via diffusion by simply allowing EWNS to move around and find bacteria on surfaces; in another method high surface charge of EWNS facilitates the targeted delivery of the EWNS on the surface of interest using an electric field thus maximizing their efficiency.

Mechanism of Bacterial Inactivation by EWNS

Since EWNS contain ROs, the mechanism of inactivation is believed to be ROs mediated. ROs can cause cell membrane destruction, DNA damage and oxidation of cell proteins. Out of these mechanisms, cell membrane destruction was found to be the primary mechanism. EWNS exposed bacteria showed destruction of cell membrane and cell wall resulting in loss of membrane integrity and shape of bacteria.

Application of EWNS in food safety

Antimicrobial potential of EWNS was assessed on a representative panel of food-related microorganisms. The researchers sprayed the surfaces of prewashed organic grape tomatoes and stainless steel chips with three lab strains of bacteria related to common food-borne pathogens: *Escherichia coli*, *Salmonella enterica*, and *Listeria innocua*. The researchers then exposed tomatoes to the EWNS at concentrations of 24,000 to 50,000 particles per cm^3 in an enclosed chamber, for time periods ranging from 30 to 90 minutes. Steel surfaces were exposed for 15 to 45 minutes. Depending on the bacterial species and type of surface, the exposure could achieve inactivation up to 4 logs (99.99% reduction) without compromising sensory quality of food or leaving chemical traces making it an ideal technology for chemical free applications (Pyrgiotakis *et al.,* 2016). Role of EWNS in air disinfection was also reported. It was found that it can kill 70-100% airborne bacteria like Mycobacteria and Serratia in 30 min. Besides, it is energy efficient and has been shown by an acute in-vivo inhalation toxicological study that apparently possesses no health side-effects when EWNS are inhaled (Pyrgiotakis *et al.*, 2014). The antimicrobial potency of the EWNS can be further enhanced by integrating electrolysis, electrospray and ionization of de-ionized water in the EWNS synthesis process resulting in three times increase in ROS content and increased antimicrobial activity (Vaze *et al.*, 2018). Huang et al., in 2021, introduced a new and innovative method for disinfecting leafy vegetables using a "dry" nano-aerosol-based antimicrobial technology. This approach involves the use of engineered water nanostructures (EWNSs) as nano-sanitizers. The EWNSs are created through a combination of electrospray and ionization processes using aqueous solutions containing active ingredients that are generally recognized as safe. Very recently Vaze *et al.* (2022) reported the efficacy of EWNS technology as a nano-carrier for delivering a minuscule dose while inactivating human corona virus, making this an attractive technology against SARS-CoV-2 .

Advantages of EWNS in food application:

- Chemical free, organic disinfection method
- Can be used across the food production and distribution chain
- Against Gram positive and Gram negative bacteria
- No effect on sensory quality
- No toxicological effects
- Can be used at normal temperature and pressure
- Cost-effective

Disadvantages of EWNS in food application:

- Only surface disinfection
- Needs smooth surface
- Cannot inactivate endospores of bacteria

Conclusion

In conclusion, the development of a novel, chemical-free method using engineered water nanostructures (EWNS) for food decontamination shows great promise. As researchers refine and scale up this technology, they aim to determine optimal conditions for maximum pathogen destruction and expand its application to various food pathogens and spoilage microorganisms. Furthermore, the versatility of this method opens doors to other potential applications such as wound healing, air disinfection, virus inactivation, and preservation of art and artifacts. While no method can guarantee complete eradication of microflora, this environmentally friendly intervention offers a "green" alternative to conventional disinfection methods, holding significant potential for the food industry and beyond.

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