Decontamination of *Bacillus subtilis* spores in a sealed package using a non-thermal plasma system

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Résumé

The safety of packaged food and medical devices is a major concern to consumers and government officials. Over the last century, numerous, alternative or complementary preservation technologies to classical (thermal) processing have been investigated. Non-thermal atmospheric plasma has been used effectively for surface decontamination. Apart from irradiation and heat, there is currently no technology which can inactivate microorganisms inside a sealed package. Using atmospheric plasma in a sealed package offers a significant breakthrough in food processing and safety technology.

A recent invention based on the principles of non-thermal, atmospheric plasma has shown the ability to reduce bacterial contamination inside a sealed package. The invention (PK-1) is based on a dielectric barrier discharge (DBD) with plate electrodes comprised of insulated conductors connected to a power unit with specifications of 18 kV @ 30 mA @ 60Hz. The package of the food is in contact with high voltage electrodes and provides dielectric resistance, thereby limiting current flow through the package and minimizing power requirements for treatment. Only 40-50 W of power are needed to ionize air inside a 4 L re-sealable plastic (LDPE) bag (Klockow and Keener, 2008). The process ionizes any gas within the electric field inside the packaged food or medical device utilizing the high voltage. Ionization can generate significant amounts of reactive molecules, including ozone concentrations above 1% in a few minutes. Only a few degrees increase in product surface temperature is observed for treatment times of minutes. Specific treatment times for targeted spore or bacterial reductions are dependent on product loading, packaging material, gas composition, and package/electrode configuration. The in-package ionization process has been demonstrated in a number common packaging materials including, cardboard, glass, LDPE, HDPE, PETE, polystyrene, rubber, Tygon, and others. A U.S. patent application has been filed on this technology. More recently, a new system design (PK-2) was built and has specifications of 130 kV at 20 mA @ 60Hz. The PK-2 system can ionize a sealed package of air with an electrode gap of 10 cm.

The objective of this study was to evaluate the PK-1 and PK-2 systems in the reduction of *Bacillus subtilis* spores using packages containing air and packages containing a MAP (modified atmosphere package) gas: 65% O_2/ 35% CO_2/ 5% N_2 treated inside and outside of a plasma field (gap space allowing), and examine the UV-Vis emission spectra for the ionization field of the PK-1 system as a point of reference in ionization characterization.

The experimental design consisted of the following parameters: 1) two voltage conditions: 13.5kV with 1.5cm electrode gap (PK-1) and 80kV with 3.2cm electrode gap (PK-2), 2) two treatment conditions: in and out of field of ionization, 3) PK-1 and PK-2 optimized treatment times for maximum ozone generation: 300 sec. (s) and 120 s, respectively, 4) temperature of 23°C, and 5) two package gas types: air and modified atmospheric package (MAP) gas: 65% O_2/ 35% CO_2/ 5% N_2 in a flexible low-density polyethylene package (LDPE plastic storage bag). Measurements included: 1) bacterial reductions of *Bacillus subtilis* var. niger (*B. atrophaeus*), 2) ozone, carbon monoxide, hydrogen peroxide concentrations using a portable Drager Measurement System, and 3) relative humidity. Spore strips (3.2 cm x 0.6 cm) containing Bacillus subtilis (1.5 x 10^6/strip) were loaded into sterile uncovered petri dishes and treated with ionization generated in packages using air or MAP gas blend. Samples were treated for 300 s (PK-1) or 120 s (PK-2) and stored at room temperature (23°C) for 24 h. Relative

humidity along with initial and final ozone, final carbon monoxide, and hydrogen peroxide concentrations were measured.

Results indicated initial and final relative humidity (RH) were 20% RH/23^{o}C and 30% RH/23^{o}C respectively. After 300 s of treatment (PK-1), ozone concentration was 3500 ppm (13.5kV/44W/1.5cm gap). After 120 s of treatment (PK-2), ozone concentrations were 10,000 ppm both in and out of ionization field (80kV/150W/3.2cm). Ozone concentrations measured < 0.5ppm or non-detect (ND) after 24 h from both systems. Carbon monoxide levels measured <10 ppm (PK-1), <20 ppm (in) and < 40 ppm (out) of ionization field of PK-2 after 24 h. Hydrogen peroxide levels measured zero or non-detect after 24 h from both systems. All treated samples showed reductions in *Bacillis* spores of greater than 2 $\log_{10}$ (PK-1) and 4 $\log_{10}$ (PK-2) after 24 h. Reductions were maintained without additional re-growth at 48h. The results indicate that the PK-1 and PK-2 ionization systems have the capacity to reduce *Bacillus subtilis* spores in an in-package treatment process.

References

[1] P. Klockow, K.M. Keener, *Safety and quality assessment of packaged spinach treated with a novel ozone generation system*. Lebensmittel-Wissenschaft und Technologie. **42** (2009) 1047.