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Plasma inactivation of microorganisms on sprout seeds in a dielectric barrier discharge

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ABSTRACT

Fresh produce is frequently contaminated by microorganisms, which may lead to spoilage or even pose a threat to human health. In particular sprouts are considered to be among the most risky foods sold at retail since they are grown in an environment practically ideal for growth of bacteria and usually consumed raw. Because heat treatment has a detrimental effect on the germination abilities of sprout seeds, alternative treatment technologies need to be developed for microbial inactivation purposes. In this study, non-thermal plasma decontamination of sprout seeds is evaluated as a promising option to enhance food safety while maintaining the seed germination capabilities. In detail, investigations focus on understanding the efficiency of non-thermal plasma inactivation of microorganisms as influenced by the type of microbial contamination, substrate surface properties and moisture content, as well as variations in the power input to the plasma device. To evaluate the impact of these parameters, we studied the reduction of native microbiota or artificially applied *E. coli* on alfalfa, onion, radish and cress seeds exposed to non-thermal plasma in an atmospheric pressure pulsed dielectric barrier discharge streamed with argon. Plasma treatment resulted in a maximum reduction of 3.4 logarithmic units for *E. coli* on cress seeds. A major challenge in plasma decontamination of granular food products turned out to be the complex surface topology, where the rough surface with cracks and crevices can shield microorganisms from plasma-generated reactive species, thus reducing the treatment efficiency. However, improvement of the inactivation efficiency was possible by optimizing substrate characteristics such as the moisture level and by tuning the power supply settings (voltage, frequency) to increase the production of reactive species. While the germination ability of alfalfa seeds was considerably decreased by harsh plasma treatment, enhanced germination was observed under mild conditions. In conclusion, the results from this study indicate that cold plasma treatment represents a promising technology for inactivation of bacteria on seeds used for sprout production while preserving their germination properties.

1. Introduction

The consumption of fresh produce increases worldwide, since it is considered an important source of nutrients and promotes a healthy lifestyle. However, inherent to such minimally processed products is the short shelf life with a high level of product loss and the risk for foodborne outbreaks, which have been increasingly attributed to such products in many parts of the world (Beuchat, 2002; National Advisory Committee on Microbiological Criteria for Foods, 1999a; Olaimat and Holley, 2012; Warriner et al., 2009). Fresh produce carries a large variety of microorganisms since it is naturally contaminated from air, dust, water and soil, as well as from animal feces and pollution during the whole production process (Warriner et al., 2009). Focusing on sprout seeds (e.g. alfalfa, clover, radish, cress, mung and soy beans) and their products, sprouting conditions (warm, humid, nutrient rich) also promote bacterial growth so that their native microbiota may strongly proliferate. This can result in microbial contaminations reported as high as 10^8–10^{11} colony forming units (CFU) per gram of seeds, which frequently include pathogens like *Salmonella*, pathogenic *Escherichia coli* or *Listeria monocytogenes* (Feng, 1997; National Advisory Committee on Microbiological Criteria for Foods, 1999b; Peñas et al., 2008). The main problem with the consumption of fresh produce like sprouts is that it is often eaten raw or only minimally processed so that pathogens are not thermally inactivated as during cooking (Olaimat and Holley, 2012). As a result, numerous outbreaks of foodborne illnesses have been associated with the consumption of sprouts (National Advisory Committee on Microbiological Criteria for Foods, 1999b; Sikin et al., 2013; Taormina and Beuchat, 1999).

Consequently, legislative authorities worldwide demand a reduction of pathogens (e.g. *Salmonella* and enterohemorrhagic *E. coli*) on seeds intended for sprout production by 5 logarithmic units (log). Currently,

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the U.S. Food and Drug Administration recommends treatment with 20,000 ppm calcium hypochlorite solution prior to germination (National Advisory Committee on Microbiological Criteria for Foods, 1999b). However, chlorine-based treatment is reported to achieve on average only 1–3 log reduction in bacteria (Sikin et al., 2013). In addition it is associated with negative health effects and environmental issues because certain organic substances can combine with chlorine to form potentially carcinogenic disinfection by-products (DBPs) such as trihalomethanes (THMs) (Van Haute et al., 2013; Yang et al., 2013). Consequently, numerous alternative methods have been investigated for decontamination of sprouts and their seeds, including chemical intervention (ozone, organic acids, electrolyzed water), biological intervention (protective cultures, bacteriocins, bacteriophages) and physical intervention (thermal inactivation, irradiation, UV light, high pressure, super critical CO_2 treatment). However, so far no treatment has achieved sufficient inactivation of pathogens on sprout seeds on an industrial scale while maintaining germination and product properties (Sikin et al., 2013). In general, it is recommended to decontaminate seeds prior to germination rather than performing sprout treatment (National Advisory Committee on Microbiological Criteria for Foods, 1999b) due to higher efficiency (Caetano-Anolles et al., 1990), lower contamination levels and to avoid embedding of pathogens into the sprout tissue (Hara-Kudo et al., 1997; Itoh et al., 1998). Moreover, sprout treatment faces the challenge to preserve optical, sensory and nutritional properties of sprouts, while seed treatment primarily requires maintaining germination properties.

A promising alternative to the aforementioned methods is plasma treatment. Plasma, commonly referred to as the fourth state of matter, is a (partially) ionized gas which contains a reactive cocktail of active plasma-generated species (charged and excited species, reactive neutrals and UV photons). In particular, so-called non-thermal plasma discharges provide the unique combination of a high reactivity, due to energetic electrons, and a low gas temperature, determined by the low energy of heavy particles (ions, neutrals), which is advantageous for the treatment of temperature sensitive substrates. The sterilizing effect of plasma, which is based on the action of the plasma-generated reactive species and their synergistic combination, has been discussed in several review articles (Boudam et al., 2006; Lerouge et al., 2001; Moisan et al., 2001). Potential applications range from decontamination of packing materials (Heise et al., 2004; Muranyi et al., 2007; Schneider et al., 2005) and medical devices (Halfmann et al., 2007; Rossi et al., 2008) over air and water depollution (Kogelschatz, 2003; Kogelschatz et al., 1997) to wound healing and cancer treatment (Fridman et al., 2008; Kong et al., 2009; Schlegel et al., 2013). Moreover, studies are available investigating the plasma decontamination of food (Misra et al., 2011; Shama and Kong, 2012) and in particular granular dry food products e.g. seeds and nuts (Basaran et al., 2008; Deng et al., 2007; Hertwig et al., 2015; Niemira, 2012; Schnabel et al., 2012a; Selcuk et al., 2008). In previous publications, we reported on the investigation of plasma treatment for decontamination of wheat grains in a low pressure plasma circulating fluidized bed reactor (Butscher et al., 2015) and an atmospheric pressure dielectric barrier discharge (Butscher et al., 2016).

Besides the inactivation of microbial contamination, successful plasma treatment also has to maintain functional product properties such as the germination ability of sprout seeds. With respect to this issue, several studies on low pressure (Bormashenko et al., 2012; Filatova et al., 2009; Jiafeng et al., 2014; Šerá et al., 2010) and atmospheric pressure (Henselová et al., 2012; Mitra et al., 2014; Stolárik et al., 2015; Zahoranová et al., 2011, 2013, 2014) plasma treatment of seeds indicate that germination and growth can even be slightly improved if plasma treatment is dosed adequately with respect to power input and treatment time. However, a decrease might be provoked if conditions get too harsh.

In this study, decontamination of various sprout seeds (mainly alfalfa) by non-thermal plasma treatment in an atmospheric pressure dielectric barrier discharge was investigated, and the effect of plasma treatment on the seed germination ability was determined. In detail, it was studied in which way seed characteristics such as their surface topology (roughness, crevices) and moisture content affect the inactivation efficiency. In addition to the plasma inactivation of the native seed microbiota we evaluated the impact of plasma treatment on artificially applied *E. coli* as a surrogate organism for Gram-negative pathogens such as *Salmonella* or pathogenic *E. coli* (e.g. EHEC or EAEC), which frequently appeared in association with sprouts during the past. Furthermore, the influence of power supply settings (voltage, frequency) and treatment time on inactivation efficiency and germination probability was analyzed. Finally, we investigated the possible influence of mechanical, electrical and thermal stress factors emanating from the experimental setup, and the mechanisms of plasma inactivation were assessed.

2. Material and methods

2.1. Materials and equipment

This study focused on the inactivation of microorganisms on seeds for sprout production by non-thermal plasma treatment. The sprout seeds used in this study comprised commercially available seeds of onion (*Allium cepa*), radish (*Raphanus sativus*), cress (*Lepidium sativum*), and two different batches (M) and (T) of alfalfa seeds (*Medicago sativa*). *E. coli* strain ATCC 8739 (DSM 1576) was used for artificial contamination of seed samples.

Plasma treatment of sprout seeds was performed in an atmospheric pressure volumetric dielectric barrier discharge (DBD), schematically depicted in Fig. 1. It consisted of two parallel aluminum electrodes with dimensions of 100 mm × 200 mm covered by dielectric barriers made of polymethylmethacrylate or polycarbonate with a thickness of 2 mm and a 5 mm gas gap in between formed by two polymeric spacers along the discharge. This lab scale setup was mounted on a horizontal vibrating table (AS200 basic; Retsch, Germany), which provoked continuous bouncing and rotation of particles to support homogeneous treatment. Argon was used as a working gas, and 5.6 nlm (norm liter per min) were dosed by means of a mass flow controller of type F-201-C (Bronkhorst, Germany). For the excitation of the non-thermal plasma fast high-voltage unipolar nanosecond square pulses (2.5–10 kHz, 6–10 kV, 500 ns) were generated with a transistor switch circuit described in detail elsewhere (Peschke et al., 2011). Compared to the standard sinusoidal waveform applied in many other studies, pulsed powering provides advantages with respect to power consumption (Laroussi et al., 2004; Walsh et al., 2006), production of reactive species (Okazaki and Nozaki, 2002; Williamson et al., 2006) and discharge homogeneity (Meiners et al., 1998; Mildren and Carman, 2001).

The gas temperature inside the DBD can be approximated using a fiber-optical probe (Wertheimer et al., 2012). For this purpose, a sensor measuring the temperature dependent band gap shift of a gallium arsenide crystal (TS2/3; Polytec, Germany) was fixed to the lower dielectric barrier in the center of the discharge and connected to an appendant spectrometer (FOTEMP-Multichannel; Polytec, Germany) to approximate the gas temperature inside the DBD during seed treatment.

In order to assess the plasma intensity, the optical emission from plasma was captured with an optical fiber (QP400-3-SR-BX; Ocean Optics, USA) connected to one of the spacers along the plasma zone (see Fig. 1) and recorded with a spectrometer (USB2000+XR1-ES; Ocean Optics, USA). The integration of optical emission over the 200–1000 nm wavelength range was taken as a measure for the plasma intensity.

The relative humidity of the processed gas was determined with a Series 1100 Hygromer (Rotronic, Switzerland). For this purpose, the probe head was inserted in the off-gas line directly after the discharge zone.