Food Control 60 (2016) 636–645

Contents lists available at ScienceDirect

Food Control

journal homepage: www.elsevier.com/locate/foodcont

Plasma inactivation of bacterial endospores on wheat grains and polymeric model substrates in a dielectric barrier discharge

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ARTICLE INFO

Article history:
Received 12 May 2015
Received in revised form
14 August 2015
Accepted 1 September 2015
Available online 5 September 2015

Keywords:
Atmospheric pressure plasma
Decontamination
Bacterial endospores
Wheat grains
Food safety

ABSTRACT

Motivated by the frequent microbial contamination of granular food products like cereal grains or sprout seeds, an atmospheric pressure dielectric barrier discharge (DBD) was developed to study plasma inactivation of microorganisms on granular materials. Wheat grains as well as polypropylene model substrates were artificially contaminated with endospores of *Geobacillus stearothermophilus* as a model organism and treated in a pulsed argon plasma discharge applying different combinations of treatment time, pulse voltage and frequency. While the treatment of polypropylene substrates resulted in an efficient reduction of microbes, wheat grains, having a rough surface and a deep ventral furrow, turned out to be more challenging to decontaminate. However, an improvement in treatment efficiency could be achieved by applying longer treatment, faster pulse frequency or higher pulse voltage. Furthermore, experiments demonstrated that endospore reduction was not caused by thermal, mechanical or electrical stress factors, but a direct effect of plasma-generated species, and chemical sputtering is supposed to be the predominant inactivation mechanism. Finally, it could be shown that functional wheat grain properties (Falling number, gluten content) are not negatively affected by our plasma treatment.

1. Introduction

Food safety is a crucial issue in food industry and decontamination an essential step in many food processing industries. Due to more restrictive food laws and higher quality expectations, food safety is also gaining in importance for cereal grains and their products. Cereal grains are naturally contaminated from air and dust, water and soil, but also from animal feces and pollution during transport and processing, so that they carry a great variety of microorganisms. Amongst others, mainly bacteria such as *Bacillaceae*, *Lactobacillaceae*, *Micrococcaceae* and *Pseudomonadaceae*, as well as molds like *Alternaria*, *Fusarium*, *Helminthosporium* and *Cladosporium* are reported to be present on cereal grains (Laca, Mousia, Díaz, Webb, & Pandiella, 2006). For instance, frequent contamination occurs from *Bacillus* spp., which deteriorate product quality due to rope spoilage (Valerio et al., 2012) and potentially produce toxins (Viedma, Abriouel, Omar, López, & Gálvez, 2011). Furthermore, pathogenic microorganisms such as enteropathogenic *Escherichia coli* and *Salmonella* are occasionally found (Sperber, 2007). Although wheat storage at a moisture level below 13% is generally considered as safe (Laca et al., 2006), microorganisms can survive in a dormant state for long periods (Eglezos, 2010). While cooking of grain products usually inactivates most pathogenic microorganisms, a study on consumer behavior revealed that many people taste raw dough and batter (Akins, 2014). In 2009 an *E. coli* O157:H7 infection in the United States was attributed to the consumption of uncooked ready-to-bake cookie dough (Neil et al., 2012), and an outbreak of *Salmonella* was also associated with the consumption of raw flour in the form of baking mixtures in New Zealand in 2008 (McCallum et al., 2013).

For comestibles, the application of conventional thermal or chemical sterilization methods is limited since many products are sensitive to heat, moisture and a variety of chemicals. With respect to the inactivation of microorganisms on wheat grains and their products a variety of potential sterilization methods have already been researched, e.g. microwave and radio frequency treatment (Nelson, 1996; Vadivambal & Jayas, 2010; Vadivambal, Jayas, & White, 2007), gamma irradiation (Azzeh & Amr, 2009; Köksel,

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http://dx.doi.org/10.1016/j.foodcont.2015.09.003

Sapirstein, Çelik, & Bushuk, 1998), low energy electrons (Hayashi, 1998; Röder et al., 2009), high intensity pulsed light (Aron Maftei, Ramos-Villarroel, Nicolau, Martín-Belloso, & Soliva-Fortuny, 2014; Oms-Oliu, Martín-Belloso, & Soliva-Fortuny, 2010), ozonated water (Dhillon, Wiesenborn, Wolf-Hall, & Manthey, 2009; Ibanoglu, 2001) and high-pressure processing (Bárcenas, Altamirano-Fortoul, & Rosell, 2010; McCann, Leder, Buckow, & Day, 2013). In summary, none of these methods is able to efficiently reduce the microbial load while retaining grain and product properties.

A promising alternative to these methods is plasma treatment. Plasma, which is occasionally referred to as the fourth state of matter, is a (partially) ionized gas and a reactive atmosphere where a variety of energetic species (charged and excited particles, reactive neutrals and UV photons) are formed mainly from collisions of energetic electrons with heavy particles (atoms, molecules, ions). These plasma-generated species can each on their own, but more efficiently in their synergetic combination, inactivate microorganisms (Fridman, 2008b; Moisan et al., 2002; Von Keudell et al., 2010). Due to the non-thermal characteristic of non-equilibrium plasma discharges (energetic electrons but cold heavy particles), these plasma processes offer the unique combination of high reactivity at moderate temperatures, which is beneficial for the treatment of temperature sensitive substrates. In general, plasma-generated species only act on the surface of substrates, so that the reduction of microbial contamination is limited to the outer surface, where most of the contamination is located (Laca et al., 2006). In return, bulk properties are not affected. Many studies on plasma inactivation are available and have been reviewed by Boudam et al. (2006); Lerouge, Wertheimer, and Yahia (2001); Moisan et al. (2001). In the domain of plasma decontamination of food, studies on e.g. bacon, pork, ham, apples, melons, mangos, lettuce, cheese, eggs and nuts are reported (Misra, Tiwari, Raghavarao, & Cullen, 2011; Shama & Kong, 2012). Furthermore, recent studies on plasma treatment of corn salad (Baier et al., 2013), fresh fruits and vegetables (Schnabel, Niquet, Schlüter, Gniffke, & Ehlbeck, 2014), as well as herbs and spices (Hertwig et al., 2014) are published.

Focusing on the atmospheric pressure plasma decontamination of grain-like food products, interesting work has been done by Deng et al. (2007), who inactivated *E. coli* on almonds by means of an atmospheric pressure DBD, where they found decimal reduction times (D-values) in the range between 5.62 s and 33.78 s, largely depending on the settings of the sinusoidal power supply (frequency, voltage). Niemira (2012) pursued the same objective using a plasma jet and reported D-values between 8.62 s and 42.86 s, depending on stand-off distance and working gas. Both studies, however, did not consider the influence of temperature, which could be the predominant inactivation mechanism in their experiments. Schnabel, Niquet, Krohmann, Polak, et al. (2012) investigated the plasma decontamination of various seeds including soft wheat grains by means of microwave plasma processed air. Except for peppercorns, *Bacillus atrophaeus* endospores could be reduced by over 5 logarithmic units within 15 min of treatment, and differences were attributed to the surface topology of seeds. While a thermal effect was excluded, the influence on seed quality was not analyzed in this study, but in Schnabel, Niquet, Krohmann, Winter, et al., 2012 where rapeseed germination was not affected by plasma treatment. Hertwig, Reineke, Ehlbeck, Knorr, and Schlüter (2015) reported the reduction of vegetative and sporulated bacteria on whole black pepper by means of a radio frequency plasma jet and a microwave-driven remote plasma similar to the system of Schnabel et al. While a 15 min plasma jet treatment achieved a reduction of *Bacillus* spp. by around 1 logarithmic unit, treatment efficiency could be improved by roughly a factor of two with the remote plasma setup. Better results were obtained for the inactivation of *Salmonella*, while the natural microflora (including endospores) turned out to be more resistant. The influence of temperature was excluded for the microwave remote location, but not discussed for the plasma jet experiments. Quality parameters were unaffected by plasma treatment.

In this study, the atmospheric pressure DBD principle, which is a non-thermal plasma discharge technique (Kogelschatz, 2003), was applied, and a discharge suitable for the homogeneous plasma treatment of granular particles (<5 mm) was constructed. The setup is, in principle, scalable to industrial dimensions, since it can be easily enlarged in its horizontal dimensions. Also, it can be inclined to enable simple particle transport by vibrating conveying. While a directed particle transport was not intended in our lab scale experiments, we used a horizontally aligned vibrating table to support homogeneous plasma treatment, which is supposed to benefit the decontamination efficiency. For the powering of the discharge we applied fast high-voltage unipolar nanosecond square pulses, which is advantageous in terms of power consumption (Laroussi, Lu, Kolobov, & Arslanbekov, 2004; Walsh, Shi, & Kong, 2006), production of reactive species (Okazaki & Nozaki, 2002; Williamson, Trump, Bletzinger, & Ganguly, 2006) and discharge homogeneity (Meiners, Salge, Prinz, & Förster, 1998; Mildren & Carman, 2001) as compared to standard sinusoidal power supplies used in most other studies. For a better understanding of plasma decontamination in our experiments, we evaluated the role of potential inactivation mechanisms. In a first step, the influence of vibration (mechanical stress), the electric field between the electrodes (electrical stress) and the role of temperature (thermal stress) on endospore viability was analyzed, which is not taken into consideration in most other studies on plasma decontamination of granular food products. Then, a closer look was taken at the role of plasma-generated reactive species. Finally, the influence of plasma treatment on functional wheat grain properties was evaluated to appraise whether plasma treatment negatively affects backing properties.

2. Materials and methods

2.1. Materials

Geobacillus stearothermophilus is a Gram-positive, non-pathogenic, endospore-forming, thermophilic bacterium, and its endospores (ATCC 7953, Merck, Germany) were used as a model organism in this study. In general, bacterial endospores are known to be one of the most resistant species and, in particular, more resistant than vegetative bacteria with respect to standard sterilization methods as well as plasma treatment (Kelly-Wintenberg et al., 1999; Scholtz, Julák, & Kříha, 2010). Due to the strong heat resistance of *G. stearothermophilus* endospores, they are frequently used as an indicator for the verification of heat sterilization processes (Viedma, Abriouel, Omar, López, & Gálvez, 2010), and they have also been used in several plasma inactivation studies (Hury, Vidal, Desor, Pelletier, & Lagarde, 1998; Kelly-Wintenberg et al., 1999; Klämpfl et al., 2012; Matsui, Ikenaga, & Sakudo, 2015; Rossi, Kylian, Rauscher, Hasiwa, & Gilliland, 2009; Von Keudell et al., 2010). Here, *G. stearothermophilus* endospores were chosen due to their high resistance against thermal impact and plasma treatment.

The focus of this study is to investigate the feasibility of plasma inactivation of bacterial endospores on wheat grains (Premium wheat, Bühler, Switzerland). In addition, polypropylene (PP) granules with an average diameter of 3.6 mm (PP Regranulat natur, Minger AG, Switzerland) were used as a model substrate to assess the influence of surface properties, and PP plates (Amsler & Frey, Switzerland) with dimensions of 76 × 26 × 1 mm were used to study the effect of the substrate shape.