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Decontamination of whole black pepper using different cold atmospheric pressure plasma applications

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ABSTRACT

Whole black pepper is a dry product, which is often naturally contaminated with bacterial endospores and sometimes also with human pathogens like Salmonella. Dry pepper itself is a shelf-stable product, but if it is incorporated into high moisture minimally processed food, the microorganisms can reduce the shelf-life of the final product and/or can cause foodborne diseases. In this study the antimicrobial effect of two different atmospheric pressure plasma applications for the decontamination of whole black pepper was investigated. Naturally contaminated peppercorns and with *Bacillus subtilis* spores, *Bacillus atrophaeus* spores and *Salmonella enterica* inoculated ones were treated using a plasma jet or a microwave-driven remote plasma. Surface color and the content of essential oils and piperine was measured. *S. enterica*, *B. subtilis* spores and *B. atrophaeus* spores were reduced by 4.1, 2.4 and 2.8 log, respectively, after 30 min remote plasma treatment. Direct plasma jet treatment did not result in equivalent inactivation levels. However, both plasma applications did not considerable affect the quality parameters.

1. Introduction

Pepper is one of the most frequently used and imported spices in the EU (CBI., 2010). In general pepper can have a high microbial load with viable counts greater than 10^7 cfu/g and most of them are spore-forming bacteria (Boer, Spiegelenberg, & Janssen, 1985; Piggott & Othman, 1993). Further, black peppercorns can also be spoiled with human pathogens like the spore-forming *Bacillus cereus* and *Clostridium perfringens*; and Salmonella (Boer et al., 1985). The hygienic handling conditions of herbs and spices, during harvest and following processes like drying, in their country of origins determines the initial level of bacterial contamination. Due to the low water activity on the seed surface the present microorganism cannot grow and multiply. However, dry stress resistant microorganisms, like bacterial spores or some types of Salmonella, are still viable and have the ability to multiply if the product is rehydrated and a sufficient amount of nutrients is available. Though contaminated pepper can cause foodborne diseases, besides being responsible for a drastical reduction of the product's shelf-life. Of special concern are ready-to-eat food products, because they are not subjected to further heat treatments (Little, Omotoye, & Mitchell, 2003). In 2010 contaminated red and black pepper, which was incorporated in salami, was the reason for a Salmonella outbreak in 44 states of the United States (Gieraltowski et al., 2013).

The current decontamination technologies for herbs and spices are thermal or chemical treatments, like steam treatments and fumigation, besides the irradiation with gamma rays (Schweiggert, Carle, & Schieber, 2007). The fumigation with ethylene oxide and the gamma irradiation are quite efficient. However the use of ethylene oxide can lead to carcinogenic byproducts and is banned by law in the European Union (Schweiggert et al., 2007; Tateo & Bononi, 2006). Irradiation can be applied only in authorized facilities and in controlled doses in order to be safe and harmless; moreover it has a poor consumer acceptance. Steam treatment is extensively used in the European herbs and spices industry. For spices with an high microbial load, like pepper, this method is not recommended due to its low reduction effect and the possible alterations in aroma and odor (Schweiggert et al., 2007; Tainter & Grenis, 2001). Therefore the development of alternative processes becomes necessary; a potential technology could be the application of cold plasma.

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Cold atmospheric pressure plasma (CAPP) is a non-thermal technology, which enables a microbial multi target inactivation on food surface. Plasma is either a partially or complete ionized gas and is the fourth state of matter. Cold plasma can be generated under atmospheric and low-pressure conditions, using radio frequency or microwave sources. Plasma applications working under atmospheric pressure have already antimicrobial effects at temperatures below 40 °C (Fröhling, Baier, Ehlbeck, Knorr, & Schlüter, 2012; Knorr et al., 2011). The generated plasma contains different reactive species, like free radicals and charged particles, furthermore heat and UV light, being responsible for the antimicrobial plasma effects (Laroussi, 2002; Moisan et al., 2002). The working gas used and other process parameters determine the concentration of these different reagents (Ehlbeck et al., 2011; Weltmann et al., 2008). Various studies showed the potential of non-thermal atmospheric plasmas to inactivate vegetative bacteria, molds and bacterial endospores on almonds (Deng et al., 2007), fresh pork meat (Fröhling, Durek, et al., 2012) or on different herbs and spices (Hertwig et al., 2015). Furthermore, the emitted reactive reagents may react with lipids, protein, carbohydrates or other food components (Schlüter et al., 2013). A detailed review about the interaction of CAPP with various food systems was given by Surowsky, Schlüter and Knorr (2014). Hertwig et al. (2015) showed a product-specific alteration of the color after non-thermal remote plasma treatment, the plasma treatment had no impact on the color of black peppercorns. In food microbiology models of inactivation kinetics are useful to describe the destruction of microbial populations, since the knowledge about the quantity of surviving microorganisms is essential to guarantee food quality. Various studies on the inactivation of *Bacillus* spores by direct plasma treatment reported biphasic inactivation kinetics (Moisan et al., 2002; Moreau et al., 2000). They assume an inactivation process depending on different mechanisms, like the inactivation due to UV light and the decomposition of the microorganism through photodesorption and etching.

The objective of this study was to investigate the antimicrobial effect of two different plasma sources for the decontamination of whole black pepper. A microbial characterization of the used whole black peppercorns was done, to determine the main spoiling microorganisms. Naturally contaminated peppercorns and with *Bacillus subtilis*, *Bacillus atrophaeus* spores and *Salmonella enterica* inoculated samples were treated. *B. atrophaeus* was chosen as the surrogate microorganism for chemical and physical inactivation processes. Furthermore the surface color, the content of volatile oils and the main aroma compound of pepper, piperine, were measured, to estimate the impact of the plasma treatment on the product quality.

2. Materials and methods

2.1. Growth of bacteria and spore preparation

S. enterica DSM 17058 was stored on cryo beads (Carl Roth GmbH, Karlsruhe, Germany) at −80 °C. One cryo bead was added to 5 ml sterile nutrient broth (Carl Roth GmbH, Karlsruhe, Germany) and incubated at 37 °C for 24 h under continuous shaking (125 rpm). Afterwards, the optical density of the pre-culture was measured at 620 nm (OD_{620}) and 25 ml of nutrient broth was inoculated with *S. enterica* cell suspension, corresponding to a start OD of 0.07. The cell culture was incubated for 24 h at 37 °C under continuous shaking (125 rpm) to achieve *S. enterica* cell cultures in the stationary phase. Each day, a fresh prepared *S. enterica* culture was used.

Bacillus spore strains used in this study, *B. subtilis* (PS 832) and *B. atrophaeus* (WIS 39 6/3), were sporulated using a method described elsewhere (Nicholson & Setlow, 1990). Sporulation was induced at 37 °C on solid 2x SG medium agar plates without antibiotics. The spore suspension was cleaned by repeated centrifugation (3-fold at 5000 *g*), washed with cold distilled water (4 °C), and was treated intermittently with sonication for 1 min. The clean spore suspensions contained ≥95% phase bright spores and nearly no spore agglomerates. The spore suspension was stored in the dark at 4 °C.

2.2. Sample preparation

Whole Black peppercorns (*Piper nigrum*) were purchased from JJ Albarracin (Murcia, Spain). 3.5 g of sterile peppercorns were placed into a sterile beaker and 175 μL (*Bacillus* spores) or 350 μL (*S. enterica*) cell suspension was added. The beaker was placed on an automatic shaker and shaken for 4 min at 400 rpm to obtain a homogenous coating of the microorganisms on the seed surface. The seeds inoculated with *Bacillus* spores were placed under a clean bench and allowed to dry for 30 min at room temperature. Salmonella inoculated samples were dried for 16 h to obtain a natural selection towards drought stress resistant one. The initial contamination of the inoculated microorganisms on peppercorns was approximately 10^7 cfu/g.

2.3. Plasma sources and plasma treatment

Two different types of plasma devices were used. A direct plasma treatment with a radio frequency (rf) plasma jet and an remote treatment with a microwave generated plasma. The rf-plasma jet equipment is described elsewhere in detail (Brandenburg et al., 2007). The apparatus consists of a ceramic nozzle (nozzle tip diameter approx. 7 mm) with a needle electrode inside, a grounded ring electrode at the nozzle outlet, an rf-generator and a gas supply system. The rf-voltage is coupled with the needle electrode. The plasma is generated at the tip of this electrode and expands into the air outside the nozzle. Depending on the gas flow rate and the application power the plasma had a length of up to 30 mm and a diameter of about 8 mm. Before the plasma treatment, the atmospheric pressure plasma jet was let run at experimental conditions for 15 min to allow preheating and passivation of the electrodes. For the treatment argon was used as working gas with a gas flow of 10 standard liter per minute (slm) and an operation power of 30 W. 1 g non-inoculated and inoculated black peppercorns were place in individual sterile petri dishes (30 mm diameter) and placed on an automatic shaker below the collimated plasma beam with a distance of 12 mm to the nozzle outlet. During the treatment the seeds were shaken continuously (at 250 rpm) to obtain a homogenous distribution of plasma on the surface layer of the seeds. The samples were treated up to 15 min.

For the remote CAPP treatment plasma processed air (PPA) was used. The PPA was generated by a microwave-driven plasma torch (PLexc®, INP, Greifswald, Germany). The microwaves had a frequency of 2.45 GHz and a power consumption of 1.2 kW. The process gas was air with a gas flow of 18 slm (standard liter per minute, 30.3975 $(Pa*m^3)/s$). The generated microwave plasma had a peak temperature of about 3700 °C. An optical emission spectrum of the used microwave plasma torch is shown by Pipa, Andrasch, Rackow, Ehlbeck, and Weltmann (2012). The plasma generating device (Fig. 1) was connected with a 25 cm long metal tube to a concentration bottle, which was connected to the reaction chamber (250 ml sterile glass bottle). During the way in the concentration bottle the PPA cooled down to 120 °C and inside further to 22 °C. 3.5 g of non-inoculated and inoculated pepper, respectively, was transferred into the reaction chamber. After filling the reaction chamber with the PPA, the bottles were shaken to obtain a