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Non-Thermal Atmospheric Pressure Plasmas for Food Decontamination

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Abstract. Plasma is used as a common technology for the treatment and modification of surfaces in a variety of industrial branches. Decontamination of inorganic materials by plasma is possible with deterioration of the materials properties of a few nanometres. A very new and innovative field of research is the application of non-thermal atmospheric pressure plasma on food for produce sanitation. The experimental set-up implements microwave plasma, which generates plasma processed air (PPA) containing manifold RNS-based chemical and antimicrobial compounds. Different agricultural produces were first contaminated with microorganisms followed by a treatment with PPA. After a post-plasma-treatment time of maximum 15 minutes with PPA reduction factors of microbiological load greater than 6 log were detected. Furthermore, germination and sensory examinations showed only little influences to the produce. The characteristics of plasma and its generated cocktail of chemical compounds leading to a high microbial inactivation on various specimens and offering a wide range of possible applications.

Keywords: Non-Thermal atmospheric pressure plasma, decontamination, food, feed, microorganisms

1. Introduction

Gentle sanitation of fresh fruits and vegetables is highly demanded since especially produce that is eaten raw increases the risk of food borne illnesses. Last noticed outbreaks in Western Europe were related to EHEC (enterohemorrhagic *Escherichia coli*) on seed/sprouts 2011, to *Listeria monocytogenes* on meat and to Norovirus in frozen strawberries 2012. Currently used disinfection or sanitation methods for fresh fruits and vegetables lack antimicrobial effectiveness, but are high in costs, water consumption or chemicals. Non-thermal atmospheric pressure plasma offers a promising opportunity for the preservation of fresh food. The antimicrobial effects of plasma are well-known and investigated [1]. Here, a microwave driven torch was studied for its antimicrobial efficacy as well as its impact on the quality of agricultural important seeds, fresh fruits and vegetables.

2. Materials and Methods

2.1. Investigated Microorganisms, Seed and Specimens

For microbiological experiments of seed *Bacillus atrophaeus* Nakamura 1989 (ATCC 9372) in the sporolated form was used in concentrations of 10^8 cfu · ml^{-1} (colony forming units per milliliter) suspended in sterile, distilled water.

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The seed of *Brassica napus*, *Raphanus sativus*, *Anethum graveolens*, *Daucus carota*, *Petroselinum crispum*, *Triticum aestivum* and *Piper nigrum* were investigated.

For microbiological experiments of fresh fruits and vegetables, *Escherichia coli* K12 (NCTC 10538), *Pseudomonas marginalis* (ATCC 10844), *Pectobacterium carotovorum* (ATCC 15713), *Listeria innocua* (ATCC 33090), *Staphylococcus aureus* (ATCC 6538), *B. atrophaeus* Nakamura 1989 (ATCC 9372), in its sporulated form, and *Candida albicans* (ATCC 10231) were used in concentrations of 10^8 cfu · ml^{-1} suspended in sterile, distilled water.

The vegetables investigated were lamb's lettuce (*Valerianella locusta*) and carrot (*Daucus carota* subsp. *sativus*); the fruits were apple (*Malus domestica*; peal and pulp) and strawberry (*Fragaria x ananassa*). The contaminated area was 2 cm × 2 cm on the outer surface of all specimens except apple pulp.

2.2. Contamination of Seed, Vegetables and Fruits

The contamination of seed was done by a self-established dipping-and-drying method, described in [2]. The seed were stored in tubes and covered with a 10^8 cfu · ml^{-1} endospore suspension for 30 minutes, followed by ambient air drying under aseptic conditions in order to eliminate all the humidity of the seed. Drying prevents the endospores from germination. Using this method, a reproducible adhesion of bacterial endospores was achieved.

The fresh-cut fruits and fresh vegetables were contaminated with the bacterial or fungal suspension by pipetting 100 µL suspension with a concentration of 10^8 cfu · ml^{-1} to the outer surface of all the investigated specimens and to the inner surface of the apple. Afterwards, the fresh products were kept under aseptic and cool conditions that the suspension could dry.

2.3. Recovery of Microbial Contamination of Each Specimen

The recovery of microbial contamination, respectively its residues on the specimen (seed, fresh food), was realized by shaking the fresh products in 10 mL or 30 mL, respectively, nutrient broth for 10 min and by using the surface-spread plate-count method with tryptic soy agar plates. The detection limit of this procedure was 10 cfu · ml^{-1} for the seed and 1 cfu · ml^{-1} for the fresh specimen, respectivily. If the number of microorganisms fell below the detection limit, i.e., no viable microorganisms has been found, these values were set at detection limit in the graphical representations.

2.4. Decontamination by Microwave Plasma Processed Air (PPA)

Non-thermal plasma treatment of the contaminated specimens was performed with microwave-driven discharge processed gas [1]-[4]. The microwaves had a frequency of 2.45 GHz and the supply power was in the range of 1.1 kW. Accordingly, the gas temperature was about 4,000 K at a gas flux of 16 slm air. The contaminated specimens were placed at the bottom of a 250 mL glass bottle. The distance between the plasma torch and the glass bottle was about 25 cm connected via a metal tube. The discharge was ignited for 7 s and the reactive gas was passed into the glass bottle. Afterwards, the bottle was closed for 5, 10 and 15 min to decontaminate the specimens. The observed inactivation of microorganisms depended on the closing and storage with long-lived reactive chemical species during post-plasma treatment time.

2.5. Germination

For studies, plasma treated and untreated control seeds were placed in petri dishes (90 mm in diameter) as transparent germination trays. Control samples consisted of seeds that had been kept under atmospheric conditions only. Seeds were transferred from glass bottle into petri dishes (10 seeds/petri dish). Each dish contained moist filter paper. The paper was maintained in a moist condition by misting with distilled water as required. Petri dishes were placed in laboratory with constant environmental conditions consisting of a temperature of 22 ℃, and a photoperiod of 16/8 day/night, respectively. The seeds were examined every 24 h for signs of germination. When radicle emergence was 0.5 cm, seeds were considered germinated. Seeds were continuously counted until the germination percentage was constant over a 72-h period.

2.6. Examination of Sensory Properties

Due to the fact that the legal classification of food treatment by physical plasma remains undetermined in Germany, the sensory examination and interpretation of the plasmatreated products occurred exclusively to texture, appearance and odor. The sensory examination was carried out as a simple descriptive test in order to obtain a first insight on possible changes in product quality after a treatment with plasma processed air (PPA). The plasma-treated products were unwashed, unwaxed and uncut apples (type Gala Royal) with reddish to yellow-green color. The examination was conducted according to DIN 10964 at 22 °C by neu.zlt-Zentrum für Lebensmitteltechnologie Mecklenburg-Vorpommern GmbH located in Neubrandenburg, Germany (DIN 10964). The simple descriptive test according to DIN 10964 is an analytical study, and therefore an objective test, in which the samples are analyzed according to certain specifications. The method is applicable for the determination of production factors and serves as a basis for the production of specific rating scales. The positive and negative features or characteristics of the test samples are described with expressions that are either arbitrary or taken from a predetermined list. Five sensory assessors examined the PPA-treated and original samples with encrypted codes. The panel did not know whether or how the apples were treated. After every third sample, a break was inserted and the sense of smell was neutralized by coffee aroma. The apples were PPA-treated under different conditions. Combinations of cooled (10 °C) and uncooled (23 °C) apples with cooled (10 °C) and uncooled (25 °C) environment as well as treatment times of 2 and 10 min were investigated.

3. Results

3.1. Decontamination of Seed by PPA

The inactivation effects of PPA were investigated with contaminated seeds of *B. napus*, *R. sativus, A. graveolens, D. carota, P. crispum, T. aestivum* and *P. nigrum.* The results of these investigations are shown in Fig. 1. All untreated seeds (reference) had a contamination of 10^6 to 10^7 cfu · ml^{-1}. Depending on the treatment time, microorganism and seed, reduction rates greater than 6 log were achieved [3]. The reduction was highest for *T. aestivum* and lowest for *P. nigrum*.

3.2. Investigations of Germination for PPA Treatment

PPA was investigated for seed germination. The plasma treatment resulted in no significant differences in seed germination from the control seed. The viability was also not negatively affected at all, resulting in a complete final germination for treated and untreated seeds (Fig. 2). However, after the first day of sowing, treatment with PPA resulted in higher germination for treated compared with control *B. napus* seeds [2].

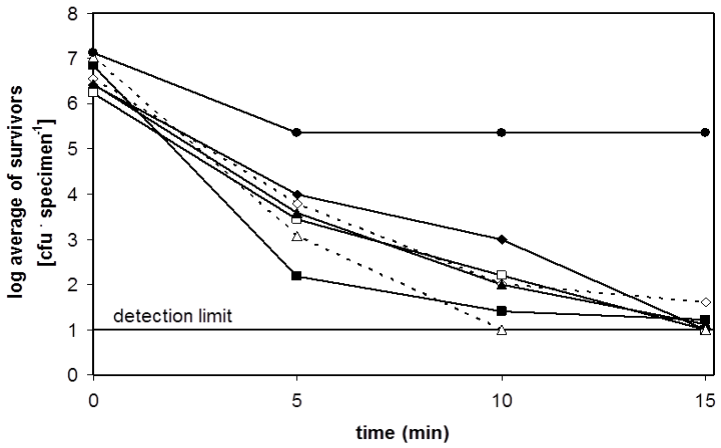

Fig. 1: Results of the plasma treatment of seeds. Seeds were contaminated with endospores of *B. atrophaeus* in concentrations of 10^8 cfu · ml^{-1}. (◆) *Brassica napus*, (◇) *Raphanus sativus*, (■) *Daucus carota*, (□) *Petroselinum crispum*, (▲) *Anethum graveolens*, (△) *Triticum aesitvum*, (●) *Piper nigrum*.

3.3. Decontamination of Fruits and Vegetables by PPA

Experimental results (exemplary apple peal – Fig. 3) showed an antimicrobial reduction of up to 6.2 log-steps depending on the specimen and microorganism [4]. As expected, differences in the antimicrobial efficiency for apple peal and pulp, respectively, strawberry, lamb's lettuce and carrots for the groups of gram-negative and gram-positive bacteria, bacterial spores and yeast were observed.

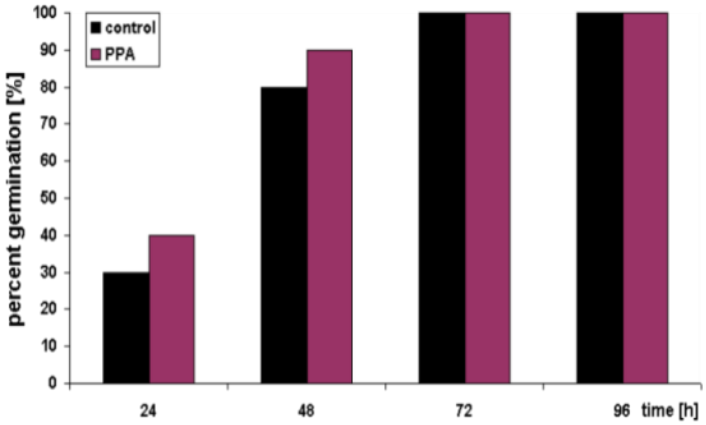

Fig. 2: Percent germination of PPA-treated and untreated control seeds of *B. napus*.

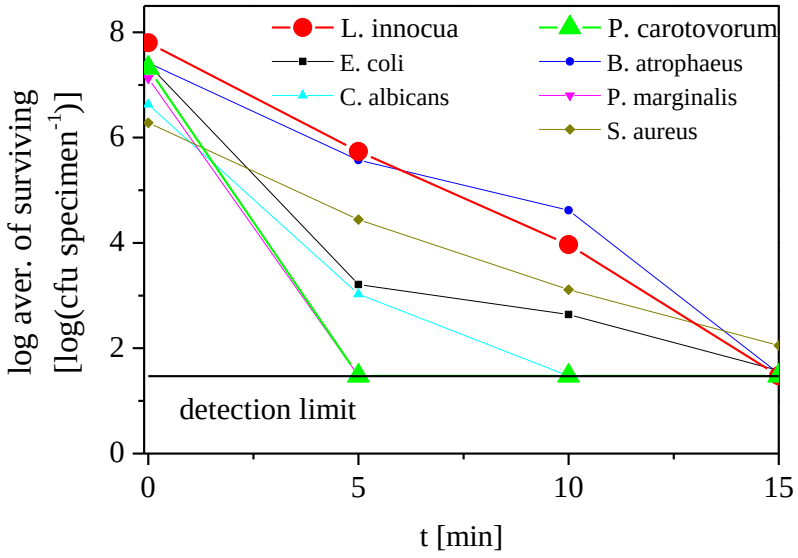

Fig. 3: Inactivation kinetics of microorganisms on apple peal as a result of treatment with PPA.

3.4. Sensory results for PPA-treated apples

The examination of sensory properties intended to determine whether using attributes for odor and appearance to distinguish between treated and untreated samples could be possible [4]. For this purpose, an assumption was made by panelists. In a rough visual observation, no obvious changes were detected in color and shape for all apples. Individual deviations are given to the respective samples in Table 1. The apples were examined at the day of the plasma treatment and 4 days later. When the panellists decided for "treated", they based their choice on odor. The texture and appearance were completely unaffected. Differences in treatment time and cooling conditions were not detected.

Table 1: Sensorical examination of apples after treatment with PPA.

temp. conditions	plasma time	odor		assessment
		0 days after	4 days after	
10 ℃, product 10 ℃, environment	0 min	sour	species specific	treated
	2 min	sour	chemical	treated
	10 min	species specific	species specific	non-treated
24 ℃, product 10 ℃, environment	0 min	species specific	chemical	treated
	2 min	species specific	species specific	non-treated
	10 min	sour	species specific	treated
10 ℃, product 24 ℃, environment	0 min	species specific	species specific	non-treated
	2 min	species specific	species specific	non-treated
	10 min	species specific	species specific	non-treated
24 ℃, product 24 ℃, environment	0 min	species specific	species specific	non-treated
	2 min	species specific	species specific	non-treated
	10 min	species specific	species specific	non-treated

4. Discussion

Non-thermal plasma treatment of foods is a promising technology in that it acts rapidly, does not leave toxic residuals on processed parts or in the exhaust gas and the temperature rise can be kept at an acceptable level [5]. The combination of plasma species with a non-thermal treatment mode makes non-thermal plasmas particularly suited for decontamination in food processing settings [6]. This process is practical, inexpensive and suitable for decontamination of products where heat is not desirable [7]. For the inactivation of *Bacillus atrophaeus* endospores and other microorganisms by microwave plasma processed air (PPA) described within, only physical stresses by chemicals and biocidal agents are important. Other stresses such as temperature, pressure or radiation can be excluded due to the experimental set-up.

Due to the plasma set-up and dry air (below 32 % relative humidity) as working gas chemical reactions and species mainly based on RNS are expected. Nitrogen and oxygen in air react to nitrogen monoxide (NO*), which further leads to the generation of nitrogen dioxide (NO_2*) with oxygen (O_2). NO* and NO_2* are two stabile radicals with known antimicrobial effectivity. Another product could be peroxynitrite ($ONOO^-$) throughout the reaction of NO* with superoxide radical (O_2*$^-$) [8], [9]. All these reactions are possible in dry air after plasma ignition. Taking into account that fresh fruits and vegetables contain high amounts of water, other chemical reactions may happen. The experiments showed a strong acidification which might be a result of the final end product of all reactions nitrous acid (HNO_2). Usually HNO_2 decays to hydrogen (H^+) and nitrite (NO_2^-), but a pH-value beneath 2.75 could lead to a spontaneous forming of OH* and NO* radicals.

Most of the mentioned ions, radicals and molecules are highly toxic for microorganisms and the chemical cocktail as well as the pH shift may result in the gained inactivation.

The investigations were focused on the decontamination of fresh fruits and vegetables, in regard of food quality after plasma treatment which should be taken into account. First publications confirmed an influence, especially when plasma is directly indicated at the product. Grzegorzewski et al. (2009, 2011) investigated the influence of a direct treatment of lamb's lettuce by an atmospheric pressure plasma jet to its phenolic profile [10], [11].

As described before the formation of RNS, especially NO*, occurs in the presented plasma set-up. The achieved microbicidal effects indicate the antimicrobial efficiency of generated RNS. It is also very interesting why microorganisms can be inactivated by ROS and RNS and the plant material is not or just slightly affected. In plants a local and systemic defense mechanism against microbicidal pathogens based on ROS and RNS already exist [12], [13].

5. Acknowledgements

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