Decontamination of black peppercorn (*Piper nigrum* L.) using microwave-generated low pressure air plasma

D. Argyropoulos*, O. Janzen, N. Krause***, G. Romano*, A. Heindl*, B. Heberle*, M. Leins**, A. Schulz**, W. Voesgen***, S. Aurich***,U. Stroth**, J. Müller***

Institute of Agricultural Engineering in the Tropics and Subtropics, Universität Hohenheim, 70599 Stuttgart, Germany
****Institute of Plasma Research, Universität Stuttgart, 70569 Stuttgart, Germany***
*****Arotop Food & Environment GmbH, Institut für Geschmacksforschung, Lebensmittel- und Umweltanalytik, 55129 Mainz, Germany***

Corresponding Author : Dimitrios Argyropoulos, Garbenstrasse 9, 70599, Stuttgart, E-mail: dimitrios.argyropoulos@uni-hohenheim.de, Tel. +49 (0)711 459 23112, Fax: +49 (0)711 459 23298.

Abstract: The preliminary results show that microwave-generated low pressure air plasmas could be a very efficient method for the decontamination of spices since the population of a microorganism (*Bacillus subtilis*) commonly found in black pepper (*Piper nigrum* L.) was significantly reduced on test substrates in a very short period of time. Based on the experimental results, a laboratory apparatus was developed for the sterilisation of spices.

Keywords: *Piper nigrum* L., non-thermal air plasma, *Bacillus subtilis*, sterilisation

1. INTRODUCTION

Black pepper (*Piper nigrum* L.) is one of the most commonly used spices worldwide. The plant is indigenous to India and cultivated in several Tropical countries such as Vietnam, Indonesia and Malaysia. Black pepper is obtained by dehydration of the unripe green pepper berries. Convective-air drying is a common method performed for medicinal plants, herbs and spices in order to reduce the moisture content to the equivalent hygienically safe water activity recommended for storage (Müller and Heindl 2006). The typical post-harvest processing of black pepper involves thermal treatment (blanching) prior to traditional sun drying followed by cleaning, grading and packaging (Bimi ***et al.*** 2004). The fresh berries are usually spread over mats and turned at regular intervals to allow uniform drying. Solar drying systems (Müller ***et al.*** 1989; Esper and Mühlbauer 1998; Heindl 2000) or convection type mechanical dryers (Heindl and Müller 1997) are also utilized for drying herbs and spices. Müller 2007 has put forward a review on convective drying of medicinal, aromatic and spice plants. Moreover, the application of microwave energy in combination with conventional hot air or vacuum drying of herbs and spices has been extensively reviewed (Heindl and Müller 2007). Correctly dried black pepper with a moisture content of less than 10% in wet basis is assessed by its characteristic pungency mainly piperine, appearance, essential oil content and microbial load (Amaladhas and Korikanthimath 2003). However, improper application of the method results in incomplete drying which causes subsequent microbial contamination of the product during packaging, storage and transport. A common problem occurring in imported dried spices from the processing countries is the presence of microorganisms which sometimes exceeds the threshold value postulated by the European Spice Association. The primary goal in the spice industry is to ensure the microbiological safety and sterilisation of the final products. Different conventional decontamination methods such as steam sterilisation, thermal inactivation, ozone, ethylene oxide and ionizing irradiation are employed on an industrial scale to prevent food spoilage caused by microorganisms. Nevertheless, the quality of the dried spices is frequently affected by heat treatments or residual chemicals. The thermal damage is typically associated with undesirable sensory changes, which constitute the material unacceptable for sale on international markets.

Particularly, although the application of steam reduces the total microbial count on herbs and spices significantly, the treatment usually causes intensive discoloration of plants and essential oil losses. An alternative method to conventional sterilisation technologies is the use of a partially ionized gas operating at low-pressure (non-thermal, "cold" plasmas) to achieve efficient inactivation of microorganisms for extreme temperature-sensitive materials like herbs and spices. The main mechanism of the plasma treatment relies on the sterilisation influence of the UV light, which causes lethal damage to the cells of bacteria (Laroussi 2005). The sterilizing efficacy of the plasma process was previously examined on polyethylene terephthalate (PET) foils sprayed by different kind of spores (Feichtinger ***et al.*** 2003). Furthermore, a work published by Schneider ***et al.*** 2005 demonstrated the easy scalability of this plasma sterilisation technique. Therefore, the objective of the present work was to design an experimental laboratory apparatus for the continuous treatment of spices by exposure them to low pressure air plasma. The physical properties of the black pepper were documented. Preliminary experiments were conducted using celullose strips and glass plates as test samples inoculated with *Bacillus subtilis* in order to evaluate the effectiveness of the technique on the microbiological sterilisation.

2. MATERIALS AND METHODS

Assessment of black pepper

Moisture content determination
The initial moisture content of the black peppercorns in (%) wet basis was measured by Karl Fisher Titration (Riedel-de Haen, Seelze, Germany). The mean value of five determinations was reported.

Water activity determination
The water activity of the black peppercorn was determined using a digital Rotronic-Hygrometer (Rotronic AG, Bassersdorf, Switzerland). The samples were finely milled in a laboratory water-cooled mill and placed in a thermostatic chamber for 30 min to allow for temperature equilibrium at 25 °C. Readings were obtained in the form of % equilibrium relative humidity (ERH) and expressed as a_w (ERH/100). The mean value of five measurements was documented.

Colour evaluation
The colour of the dried black peppercorn was evaluated using a Minolta Colorimeter (CR-400 Minolta Co., Ltd., Osaka, Japan). The instrument was calibrated with a standard white tile at D_{65} illumination before taking measurements (Y=93.7, X=0.3158, y=0.3324). Twelve readings were performed on the surface of the sample by placing the colorimeter head directly above the peppercorn. The measurements were replicated three times and the average value was reported. The colour parameters were expressed as L* describing lightness (L*=0 for black, L*=100 for white), a* describing intensity in green-red (a*<0 for green, a*>0 for red), b* describing intensity in blue-yellow (b*<0 for blue, b*>0 for yellow). Furthermore, other colour components such as Chroma (C*) and hue angle (h*) were also calculated.

Size and bulk density
The size of the peppercorns was measured by an electronic Vernier calliper at an accuracy of 0.01 mm and the mean value of 100 peppercorns was obtained. The bulk density of the black pepper was determined using a container of defined volume (1000 cm^3) filled with material and the weight was recorded. The mean value of five replications was taken.

Texture measurement
A texture analyzer (Instron Universal Testing Machine, Model 4301, Norwood, USA) was used for the compression tests of black pepper. The trials were carried out at room temperature (25 °C). A 8 mm diameter needle probe was used to puncture an individual peppercorn placed over a plate. The crosshead speed was maintained at 100 mm/min. Twelve measurements were performed per batch of samples. The test was repeated three times and the average value was documented.

Experimental apparatus
Part of the work was the design of an experimental apparatus on laboratory scale for the decontamination of spices.

Preliminary tests using Planartron

The preliminary experiments were carried out using the Planartron at the Institute of Plasma Research, Universität Stuttgart, Germany. The designed nominal power output of the installation is 4000 W at an operating frequency of 2.45 GHz. The microwave travels via a half coaxial wave guide, which is free to fluctuate in the low pressure region and is separated by a quartz plate from the normal pressure zone. By the use of this arrangement the microwave is homogeneously fed through the quartz

plate to form homogeneous plasma at the surface of the quartz plate in the low pressure zone.

Experimental procedure

As test samples, cellulose strips and glass plates homogeneously sprayed with 10^6 and 10^7 spores of *Bacillus subtilis* respectively were treated by low pressure air plasma. As feed gas ambient air was selected. The UV light emission was monitored between 175 and 500 nm. The samples were exposed for about 40 sec to a mean power of 400 W in pulse mode (24/206 ms). Immediately after each experiment the plasma-treated samples were packed in polyethylene bags and transported to the Arotop Food and Environment GmbH, where the sterilising efficacy of the technique was evaluated by counting the surviving colony-forming units (cfu). Furthermore, the surface structure of the test material was examined using a scanning electron microscope.

3. RESULTS AND DISCUSSION

Spectral analysis - the role of UV light

The spectrum of the laboratory microwave generated low pressure air plasma was measured by a spectrometer and the peaks of the molecules and atoms of air were assigned. Figure 1 shows a plot of relative intensity as a function of wavelength ranging between 175 and 500 nm at 0.7 mbar operating pressure.

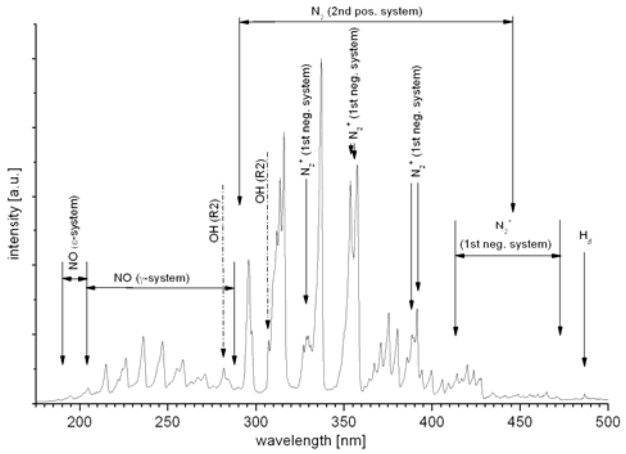

Fig. 1. Spectrum of the laboratory air-plasma at an operating pressure of 0.7 mbar

The bands in the region of 200-280 nm correspond to the gamma-system of the nitrogen monoxide (NO). Furthermore, the formation of OH-radicals in the plasma can be observed in the spectral range between 281 and 304 nm. The specific emission bands of N_2^+-radicals are just below the visible light spectrum at wavelengths of 350, 385 and slightly above 410-470 nm. In the range of wavelength between 300 and 400 nm the radiation of the molecular nitrogen dominated the plasma, however, this energy was not enough to cause serious damage to DNA in spores of *B. subtilis*.

Spore reduction kinetics of *Bacillus subtilis*

The inactivation kinetics was evaluated at an operating pressure of 0.7 mbar. Plasma was generated above the test substrates with a defined initial contamination of 10^6 spores. Figure 2 shows the changes in the population of *B. subtilis* presented in logarithmic scale and plotted against the time of the treatment. A lower value than 10^0 indicates complete elimination of the microorganisms on the cellulose strips. The mean values along with the standard deviations of five parallel measurements per pressure were taken into consideration. The plasma treatment shows a very fast inactivation kinetic behaviour and the microbial load was significantly reduced.

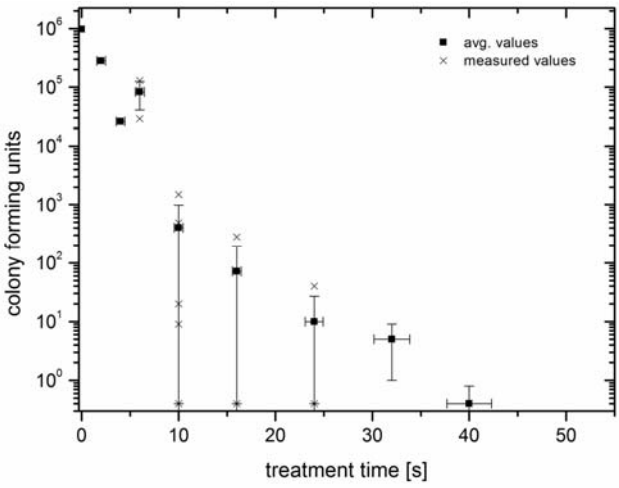

Fig. 2. Reduction kinetics of *B. subtilis* exposed to air-plasma at an operating pressure of 0.7 mbar

At the operating pressure of 0.7 mbar in pulse mode, the time required to reduce the initial microbial load (10^6) to a value of 10^1 was 24 sec. In general, a standard microorganism inactivation is equivalent to a reduction of six orders of magnitude. Due to the complex cellular structure of herbs and spices, the impact of this technique on uniform inactivation of microorganisms was also examined by exposing different sides of the test material to the microwave-generated low pressure air plasma at a pressure of 0.7 mbar. The inactivation kinetics of *B. subtilis* using a different arrangement of the strips is shown in Figure 3. A treatment time between 35 and 40 s was required for a 6-log reduction on both sides of the test substrate. No significant difference of the

sterilising efficacy of the treatment on two different sides of the test material was observed. Consequently, the results indicated the effectiveness of the laboratory air plasma to reduce significantly the population of *B. subtilis* on both sides of the cellulose strips.

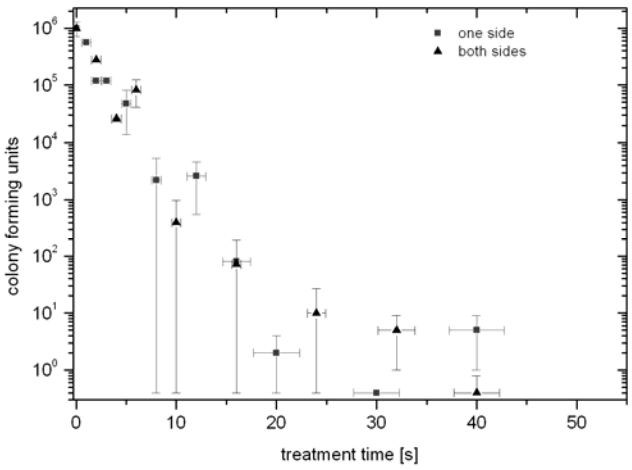

Fig. 3. Reduction kinetics *B. subtilis* after subsequent air-plasma treatment on both sides of the cellulose strips

Visual inspection of the cellulose strips

In order to evaluate whether or not a homogeneous contamination occur on the strips, the surface structure of the material was investigated using a scanning electron microscope. The cellulose strips were also examined to determine any complexity or accumulation of the microorganisms which may adversely affect decontamination. Figure 4 shows the complex structure of the individual fibres on the strip tested. It is worth mentioning that due to the light fibres of the strip and the transparency of the spores it was difficult to detect bacteria using an optical microscope.

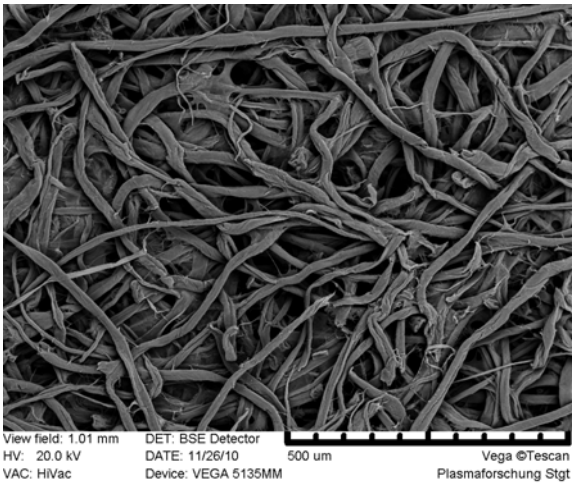

Fig. 4. SEM-images of the cellulose strips (1 mm)

Although clusters of bacteria were not recorded, a particle with a size similar to a spore was observed using a scanning electron microscope which was apparent at a higher magnification (Figure 5).

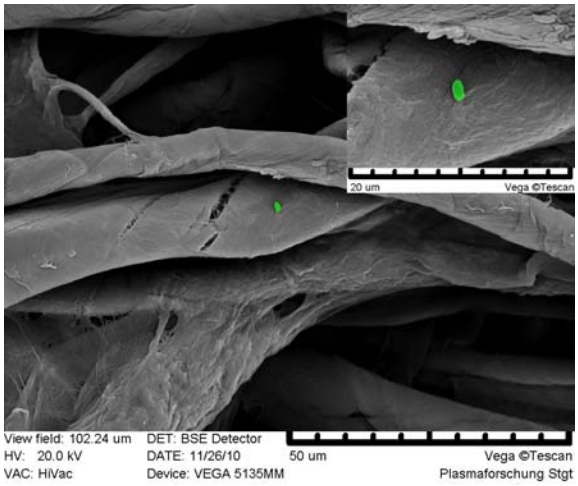

Fig. 5. SEM-images of the cellulose strips (10 µm)

Experimental laboratory apparatus

The laboratory apparatus for the microbial decontamination of different spices such as black pepper, paprika flakes and parsley was designed and constructed at the Institute of Agricultural Engineering, Universität Hohenheim, Stuttgart (Germany). Figure 6 shows a schematic diagram of the experimental installation with its components. The laboratory system essentially consists of a chamber made of stainless steel (i), a drum (ii), a motor (iii), two linear plasma sources (iv) and an infrared pyrometer (v).

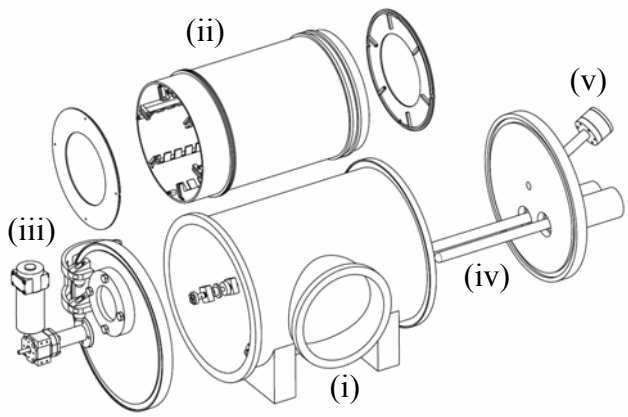

Fig. 6. Scheme of the experimental system with its components

The sample is evenly distributed in a cylindrical drum made of stainless steel with a volume of 0.01 m^3 which is rotated on its horizontal axis in the chamber where two linear plasma sources are mounted. To ensure uniform decontamination of the material six mixing heads are attached across the sample holder. Loading or discharge of the material

is facilitated using a radial gate on the left side of the chamber. The feeding device is mounted on guide rails in the main body with a carriage for easy rotation. By means of a magnetic clutch driven by a motor (Maedler, Stuttgart, Germany) the drum positioned inside the sealed chamber is rotated. One half of the clutch is attached to the motor and the other to the driven shaft.

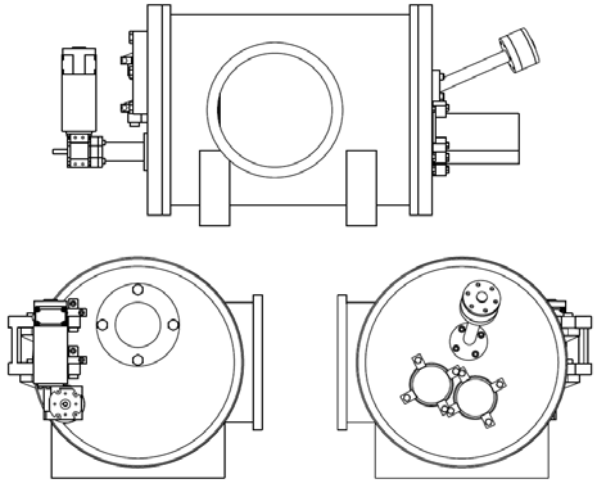

Fig. 7. Laboratory apparatus for sterilisation of spices

The operating low pressure is generated by a pump connected through a flange to the main body of the sealed chamber. The surface temperature of the product can be continuously measured during the plasma treatment by an infrared pyrometer installed in the right side of the main body (Ahlborn, Holzkirchen, Germany). The laboratory apparatus for the continuous sterilisation of spices is show in Figure 7.

Quality of black pepper

Black pepper (*Piper nigrum* L.), supplied by the Moguntia Food Service GmbH, Mainz (Germany) was evaluated in terms of different criteria. Results of size, bulk density, moisture content, water activity, texture and overall colour of the peppercorns are summarized in table 1.

Table 1. Properties of black pepper

Moisture content (% w.b.)	Water activity (-)	Size of corn (mm)	Bulk density (g/cm^3)	Texture (N)
8.92±0.32	0.44±0.01	5.04±0.27	0.57±0.01	80.23±6.31
Colour parameters				
L*	a*	b*	C*	h*
21.40±1.58	3.63±0.85	7.84±1.31	8.65±1.5	65.32±3.02

Further experiments will be conducted to investigate the influence of plasma treatment on the physical and chemical quality attributes of black peppercorns.

4. CONCLUSIONS

The preliminary results show that microwave-generated low pressure air plasmas could be a very efficient method for the decontamination of spices since the population of a microorganism (*B. subtilis*) was significantly reduced on test substrates in a very short period of time. An experimental laboratory apparatus was developed for the continuous plasma treatment of spices. Further experiments will be conducted to optimize the system by sterilising samples of black pepper, paprika flakes and marjoram.

5. ACKNOWLEDGEMENT

The authors express their gratitude to the Bundesministerium für Wirtschaft und Technologie (BMWi) for the promotion of the project (support code: KF2604701SK0) by financial support through the "Zentrales Innovationsprogramm Mittelstand (ZIM)".

6. REFERENCES

Amaladhas, H. and Korikanthimath, V.S. (2003). Processing and quality of black pepper - a review. *Journal of Spices and Aromatic Crops*, 12 (1), 1-13.

Bimi, G.B., Raj, M.A.; Kumar, A., Amaldhas, H. and Sarma, Y.R. (2004). Changes in microbial load of black pepper (*Piper nigrum* L.) during processing. *Journal of Food Science and Technology*, 41 (1), 77-79.

Esper, A. and Mühlbauer, W. (1998). Solar Drying-An Effective Means of Food Preservation, *Renewable Energy*, 15, 95-100.

Feichtinger, J., Schulz, A., Walker, M. and Schumacher, U. (2003). Sterilisation with low-pressure microwave plasmas, *Surface and Coatings Technology*, 174-175, 564-569.

Heindl, A. and Müller, J. (1997). Trocknung von Arznei- und Gewürzpflanzen. *Zeitschrift für Arznei- und Gewürzpflanzen*, 2 (2), 90-97.

Heindl, A. (2000). Solare Warmlufttrocknung von Arznei- und Gewürzpflanzen. *Zeitschrift für Arznei- und Gewürzpflanzen*, 5 (2), 80-88.

Heindl, A. and Müller, J. (2007). Microwave drying of medicinal and aromatic plants. *Stewart Postharvest Review*, 4, 1-6.

Laroussi, M. (2005). Low temperature plasma-based sterilisation: Overview and State-of-Art. *Plasma Processes and Polymers*, 2, 391-400.

Müller, J., Reisenger, G., Kisgeci, J., Kotta, E., Tesic, M. and Mühlbauer, W. (1989). Development of a greenhouse-type solar dryer for medicinal plants and herbs. *Solar and Wind Technology*, 6 (5), 523-530.

Müller, J. and Heindl, A. (2006). Drying of medicinal plants. In Bogers, R.J., Craker, L.E., Lange D. (ed), *Medicinal and aromatic plants-agricultural, commercial, ecological, legal, pharmacological and social aspects*, 237-252. Springer-Verlag, Berlin, Heidelberg (Germany).

Müller, J. (2007). Convective drying of medicinal, aromatic and spice plants: a review. *Stewart Postharvest Review*, 4 (2), 1-6.

Schneider, J., Baumgärtner, J., Feichtinger, J., Krüger, J., Muranyi, P., Schulz, A., Walker, M., Wunderlich, J. and Schumacher, U. (2005). Investigation of the practicability of low-pressure microwave plasmas in the sterilisation of food packaging materials at industrial level. *Surface and Coatings Technology*, 200, 962-966.