Effect of atmospheric cold plasma (ACP) on *Escherichia coli*, *Listeria monocytogenes* and *Salmonella enterica* serovar Typhimurium on ready-to-eat mortadella-type sausage

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Abstract – This study focuses on the use of atmospheric cold plasma (ACP) technology to reduce microbial load on mortadella-type sausage slices. Low working temperatures in combination with short treatment times (0, 30, 60, 120 seconds) at atmospheric pressure showed different effects on the inactivation of *Salmonella enterica* serovar Typhimurium (*S*.T.), *Escherichia coli (E.c.)* and *Listeria monocytogenes (L.m.)*. *In vitro*-tests on agar plates revealed a significant reduction of averagely 4.54 ± 0.54 log_{10} steps of *L.m.* owing to plasma exposure for 180 seconds. In contrast, the effectiveness of ACP treatment on microbial inactivation on mortadella was limited. The maximum inactivation for *S*.T. was 0.3 log_{10} steps, whereas the bacterial counts of *L.m.* and *E.c.* remained unchanged. Both, ACP-treated and non-treated samples were stored for 1, 7, 14 and 21 days. Microbiological analysis was carried out for all storage periods. After a plasma treatment for 120 seconds and storage over 21 days counts for *L.m.* as well as *E.c.* were significantly ($P < 0.05$) lower compared to a 30 seconds treatment (6.58 ± 0.22 to 6.25 ± 0.19 lg CFU/g and 5.63 ± 0.41 to 5 ± 0.53 lg CFU/g, respectively).

Key Words – foodborne pathogenic bacteria, cold plasma, mortadella

I. INTRODUCTION

A rising demand of fast available food of animal origin leads to an increased need of ready-to-eat products. These prepackaged deli meats, such as sliced mortadella-type sausages, are sensitive foods due to microbiological recontamination during slicing and packaging [1]. Previous non-thermal treatment approaches (use of aromatic acids, packaging in a modified atmosphere) have not lead to the desired results. Atmospheric cold plasma (ACP) is described as an emerging non-thermal technology to reduce pathogenic microorganisms on food. The ability of ACP as a fast treatment while being chemical and water free, environmentally friendly and safe for operators [2] offers the possibility for its use to extend the shelf-life of food [3]. Plasma is generated while a process gas passes through an electric field. As soon as high energy levels are achieved, intramolecular and intra-atomic structures break down, liberating free ions and electrons, while the ambient air is ionized. Collisions of these electrons produce reactive species (e.g. oxygen and nitrogen radicals), which interact in synergy with UV-radiation and charged particles with the membranes of cells and their DNA, thus, causing damages in proteins, lipids and nucleic acids [4,5].

The impact of ACP treatment on emulsion-type sausages is largely unexplored. The aim of this study was to examine the antimicrobial effect of ACP for the inactivation of microorganisms on sausage slices, during 21 days of storage.

II. MATERIALS AND METHODS

2.1 Evaluation of the method on agar plate cultures

To verify the functionality of the plasma device, initial quantitative tests were carried out with *Listeria monocytogenes* (DSM No. 19094) according to ISO 11290-1 on OCLA agar (Oxoid GmbH, Wesel, Germany). The strain was cultivated in Brain Heart Infusion (BHI) broth (Merck KGaA, Darmstadt, Germany) for 24 hours at 37 °C to achieve an estimated cell count of 10^8 cells/ ml. One hundred µL-aliquots of a serial dilution (10^{-1} to 10^{-8}) were plated on the surface of agar plates and air dried. ACP treatment was applied for 0, 10, 20, 40, 60 or 180 seconds.

Inoculation and treatment of mortadella

The main experiment was performed applying *Salmonella enterica* servoar Typhimurium (*S*.T.), *Escherichia coli (E.c.)* and *Listeria monocytogenes (L.m.)* as indicator microorganisms. Bacterial strains were obtained from the Leibniz Institute DSMZ (German Collection of Microorganisms and Cell Cultures, Braunschweig, Germany [DSM Nos. 14221, 1103, 19094]). One colony of each germ was transferred to Brain Heart Infusion (BHI) broth (Merck KGaA, Darmstadt, Germany) and incubated at 37 °C for 24 hours resulting in a final bacterial count of approximately 10^8 CFU/ml. Sliced, packaged emulsion-type sausage, type mortadella was purchased from a local super-market. Mortadella slices were wrapped, in sixes, in plastic traces in 30 g quantities and represented one sample unit. 100 µL of the bacterial suspension (obtaining approx. 10^6 CFU/g sausage) was used to inoculate the six sausage slices of one package. Afterwards, samples were ACP treated (0, 30, 60 and 120 seconds). The ACP system used in the present study was generated by the FlatPlaSter 2.0 (terraplasma GmbH, Garching, Munich, Germany [U = 18 kV, f = 12.5 kHz, P = 0.5 W/cm2]). The plasma device consists of an electrode for the plasma production, which is embedded in a plastic box and covered by a dielectric material and a grounded mesh. Prepared samples were placed within the ACP generation field, measures 127.71 x 85.43 mm (comparable to the dimensions of a 96 MicroWell plate). The distance between the sample and the plasma electrode was set to 6 mm. The study was conducted with threefold repetition for every microorganism, treatment time and storage day, performed in two sessions (n = 6). Subsequent to ACP treatment samples were sealed under high nitrogen gas flush (70 % N_2, 30 % CO_2) and stored at 4 °C ± 0.5 °C.

2.2 Microbiological analysis

Microbiological examination was conducted with three inoculated sample units for each treatment time and each bacterial strain on storage days 1 (day after ACP application and packaging), 7, 14 and 21. Standard microbiological methods were used according to ISO 6579 (*S*. Typhimurium), ISO 11290-1 (*L. monocytogenes*) and ISO 16654 (*E. coli*). The number of microorganisms was evaluated and is expressed as $\log_{10}$ CFU/g. Generally, the survival ratio of microorganisms S was defined as:

$S = \log_{10} (N_t/N_0)$

where N_t represents the number of surviving microorganisms after a session of ACP treatment, and N_0 is the initial number of microorganisms.

III. RESULTS AND DISCUSSION

3.1 ACP treatment of microorganisms on agar plates

In vitro-tests on the surface of agar plates revealed a clear reduction of at least 4.54 ± 0.54 $\log_{10}$ steps of *L.m.* owing to plasma exposure for 180 seconds. This is in accordance with the results of Lee *et al.* [3].

3.2 Effect of ACP treatment on emulsion-type sausage slices

The effectiveness of ACP treatment on microbial inactivation on mortadella was limited. The maximum inactivation for *S*.T. was 0.3 $\log_{10}$ steps, whereas the bacterial counts of *L.m.* and *E.C.* remained unchanged. Within the first two weeks of storage, no significant disparities of the microbial count of both *L.m.* and *E.c.* were observed. However, after a storage period of 21 days, significant differences of 0.33 $\log_{10}$ steps (*L.m.*) and 0.63 $\log_{10}$ steps (*E.c.*) between 30 and 120 seconds of ACP treatment occurred. *S*.T. was generally more sensitive to ACP treatment than the other bacteria studied. During the storage period, samples treated with ACP showed at each storage day constant lower counts of *S*.T. compared to the untreated control. However, it has to be emphasized, that the differences in counts of *S*.T. were likewise rather small. A significant reduction ($P < 0.05$) was observed within the first two weeks. Viable cells of *S*.T. were reduced by 0.3 $\log_{10}$ steps after 120 seconds exposure to ACP. It seems that microbial inactivation due to plasma technology was more dependent on the matrix and bacterial species than on the exposure time of ACP. However, there was a tendency of cell count decrease over storage period. Important impact factors on the efficacy of the ACP treatment in addition to the cell characteristic seem to be the

sample matrix surface, system and process parameters [6]. The greatest antibacterial effects were observed for those matrices having the most homogenous surface. This observation is in accordance with Ziuzina *et al*. [7], who reported that porous food surface offers many niche areas for bacteria, providing protection against ACP treatment. Furthermore, some authors assume that Gram-positive bacteria are less sensitive to ACP treatment than Gram-negative bacteria [8-10]. We have also been able to confirm this in the present study. *L.m.* as a Gram-positive bacterium is characterized by a thicker outer membrane in comparison to Gram-negative *S*.T. and *E.c.*. Possibly plasma reactive species can diffuse through the thinner cell wall of Gram-negative bacteria more easily, resulting in stronger antimicrobial effects. Furthermore, *L.m.* as a psychrophilic bacterium has the advantage to multiply at a broad temperature range and could possibly grow continuously over the 21 days of storage at 4 ± 0.5°C (refrigerator) [11]. The inactivation process due to plasma technology is also limited by the initial cell count in the product [12]. Generally, high initial cell counts clearly above the detection limit of the assays used for their enumeration can ensure accurate analysis of the inactivation of native microorganisms [13]. However, high concentrations of microorganisms as performed within this study possibly resulted in several clumps of bacteria that then supposedly protected each other against ACP treatment. Yu *et al*. [14] concluded that the penetration of plasma species and treatment effectiveness is affected by high surface concentrations of cells. High fat (20 %) - and protein (13 %) content of the mortadella-type sausage slices may also provide a barrier against ACP treatment. Furthermore, better results might be achieved using a plasma device with higher power supply, resulting in higher electron and ion density, in synergy with ultraviolet photons. This could lead to a more effective destruction of bacterial cells [15]. One of the criteria that may be decisive for effectiveness of ACP treatment might be the used gas. Some authors considered that N_2+O_2 mixture is more effective to reduce bacteria on meat surface [3] than only the use of the ambient air. A study by Critzer *et al.* [16] showed that oxidation of compounds only occur at the food surface, since plasma reactive species do not penetrate into the food. Consequently, ACP treatment has to be regarded as a superficial non-thermal technology that only affects microorganism on the matrix surface. Comparing the results of *in vitro* assays on agar plates with bacterial count of mortadella slices using the same bacterial strains within our study, conventional detection methods seem to be unsuitable to evaluate the efficiency of surface decontamination. Perhaps standard ISO methods have to be modified, for example using swab method to determine bacterial count as already done by Ahlfeld *et al.* [17] for virus detection.

IV. CONCLUSION

In conclusion, we confirm the potential of CAP treatment to reduce the germ content of microbial cultures on agar plates, whereas the effect on emulsion-type sausages was only limited. However, the maximum inactivation ratio was 0.3 $\log_{10}$ steps, achieved for *S*.T. Thus, *S*.T. was more sensitive to ACP treatment than *E.c.* and *L.m.*. Future studies will be necessary to evaluate the effect of a plasma device with higher power supply and a lower bacterial count on other matrices. Whether this technology leads to changes in the sensory quality, including flavor and odor, remains to be determined.

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