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THE INFLUENCE ON NONTHERMAL, LOW-PRESSURE PLASMA ON MICROBIOLOGICAL CONTAMINATION OF FOOD

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Abstract

Among many methods for food decontamination, high hopes are associated with nonthermal, low-pressure plasma technology. This study aimed to estimate the survival rate of *Escherichia coli*, *Salmonella* Senftenberg W775 and *Enterococcus faecalis* exposed to the action of plasma for the time from 0 to 800 s. As carriers there were used the laurel leaves. An analogous experiment was performed with the use of UV-C radiation. The results show that the plasma technology is more effective in bacteria reduction than ultraviolet radiation. The cells of *E. coli*, were the most susceptible to the action of nonthermal plasma and their numbers after 800s decreased by 7 log, after the same time the amount of enterococci decreased by about 6 log. *Salmonella* cells were characterized by a very high resistance to low-pressure plasma. Results of this study show that the plasma technology should be used for a longer time and in a higher dose to guarantee the full decontamination of food.

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WPŁYW NISKOTEMPERATUROWEJ, NISKOCIŚNIENIOWEJ PLAZMY NA MIKROBIOLOGICZNE ZANIECZYSZCZENIE ŻYWNOŚCI

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Słowa kluczowe: niskotemperaturowa plazma, żywność, *Salmonella* sp., *Enterococcus* sp., *Escherichia coli*.

Abstrakt

Spośród wielu metod dekontaminacji żywności duże nadzieje wiąże się z technologią zimnej, niskociśnieniowej plazmy. Celem pracy było zbadanie przeżywalności bakterii *Escherichia coli*, *Salmonella* Senftenberg W775 oraz *Enterococcus faecalis* poddanych działaniu plazmy w czasie od 0 do 800 s. Jako nośniki bakterii wykorzystano liście laurowe. Wykonano także analogiczne doświadczenie z wykorzystaniem promieniowania UV-C, aby ocenić skuteczność metody.

Wyniki wskazują, że technologia plazmowa jest efektywniejsza w redukcji liczby bakterii niż promieniowanie ultrafioletowe. Na działanie zimnej plazmy najwrażliwsze były bakterie *Escherichia coli*, których liczebność po 800 s zmalała o 7 log, po tym samym czasie liczba paciorkowców kałowych obniżyła się o ok. 6 log. Pałeczki z rodzaju *Salmonella* charakteryzowały się bardzo dużą opornością na niskociśnieniową plazmę. Wyniki badań wskazują, że produkty spożywcze powinny być poddawane działaniu niskotemperaturowej plazmy przez dłuższy czas lub generatorem o większej mocy, by uzyskać ich pełną dekontaminację.

Introduction

Consumers more often look for fresh or low-processed products, therefore it is essential to ensure their high quality and microbiological purity. Unfortunately, the growing number of food borne diseases and poisonings indicates that traditional decontamination technologies are insufficient (BIBEK 2004, *Microbiological hazards*... 2008). Pasteurization and sterilization considerably reduce the number of undesirable microorganisms, but they change the nutritive and sensory properties of food products. Competition on the market and the growing level of consumer knowledge and legal regulations force the producers to develop new technological solutions which could be used in food processing. One of more modern methods is nonthermal plasma (AFSHARI and HOSSEINI 2014). It is effective at the ambient temperature and does not increase the product temperature, which is of utmost importance, especially in the case of fresh food products. Apart from the high effectiveness of inactivation of many pathogens and spores, plasma is also environment friendly and it does not change the organoleptic and sensory values of products (ZHANG et al. 2014). Nonthermal plasma may be used to purify fresh and processed fruit and vegetables, meat, eggs or dried food products. It also gains recognition in the package industry, due to providing the appropriate storage conditions (KNOERZER et al. 2012, SCHNABEL et al. 2015). After treatment with nonthermal plasma, the nutritive and sensory values of food remain unchanged, also no presence of toxic structures was observed, which affects the maintenance of consumer safety (FERNÁNDEZ and THOMPSON 2012, KNOERZER et al. 2012). High reactivity of chemical particles being plasma components have destructive effect of biological structures of microorganisms. The highest lethality is provided by the hydroxyl radical and atomic oxygen, which affect the oxidation of protein and lipids of the cell membrane (WIKTOR et al. 2013). Pores are generated through which charged particles penetrate into the microbial cell and induce disturbances of enzyme and protein activity. Cytoplasm leaking from the generated pores may be the cause of cell death. The study conducted over nonthermal plasma has confirmed the ability to eliminate microorganisms, even those resistant, such as: bacterial spores, fungi, *Vibrio parahaemolyticus*, *Salmonella* spp., EHEC strains of *Escherichia coli*, *Staphylococcus aureus* or *Listeria monocytogenes* (PIGNATA et al. 2014, GUO et al. 2015).

The aim of this study was to evaluate the effect of nonthermal low-pressure plasma on the survival rates of the bacteria: *Escherichia coli, Enterococcus faecalis*, and the strain *Salmonella* Senftenberg W775, known for its resistance to various physicochemical factors. Carriers imitating dry food products were inoculated with microorganisms, they were subjected to the action of plasma and the number of bacteria after different times of exposure was determined.

Materials and Methods

Microorganisms used in this experiment, *Escherichia coli, Enterococcus faecalis,* and *Salmonella* Senftenberg W775, are derived from pure culture collections of the Institut f*ür* Umwelt und Tierhygiene sowie Tiermedizin, Universit*ät* Hohenheim in Stuttgart. The first two mentioned are the faecal, commensal bacteria, while *Salmonella enterica* ssp. *enterica* serovar Senftenberg strain W775 (*Salmonella* Senftenberg W775) is very common in animals and food. Additionally, it is known for its resistance to many physicochemical factors, such as high temperature, gamma radiation, dessication or disinfectants. Therefore, this bacterium is often used for the estimation of the effectiveness of different antimicrobial methods. All bacteria represent the wide range of microorganisms that may contaminate food. The bacteria were inoculated on liquid media (enriched broth, Merck 7882) and incubated for 24 hours at 37ºC. Laurel leaves were used as bacteria carriers imitating food products. They were rinsed twice in distilled water, dried, cut into squares of 1 cm, and then attached to the microscopic slides with double-sided adhesive tape. Fragments of leaves were contaminated with the bacterial suspension (100 µl each), dried in the laminar chamber and exposed to the action of nonthermal plasma. The plasma was generated in a cylindrical chamber of the diameter 100 mm and the length of 270 mm between two parallel electrodes using plasma activator FEMTO (Diener Electronic, Germany). Discharges frequency and nominal power of this activator were 40 kHz and 100 W, respectively. The chamber was evacuated to a pressure of about 0.2 mbar over approximately 5 min, before admitting the gas (air) with the rate of 5 sccm for 5 min until the desired pressured was reached and stabilized. The plasma was then ignited, at the required power, and exposure was controlled with a build-in electronic timer. To recover the bacteria, the carriers were transferred to 9 mL 0.85% NaCl and shaken for 5 min (Vortex Shaker QL-866, 2250 r/min). Suspensions were diluted (10^{-2}-10^{-8}), inoculated on suitable selective media and incubated at 37°C for 24 hours. The experiment was conducted in five replications. To perform microbiological analyses, we used the following culture media: ENDO (Merck 104044) for *Escherichia coli*, BPLA acc. to Kaufmann (Merck 7236) for bacteria from the genus Salmonella and kanamycin, esculine and azide agar for *E. faecalis* (Merck 5222).

To compare the efficacy of the nonthermal plasma technology with the effect of more commonly used in food industry ultraviolet radiation, analogous carriers were irradiated with UV-C (in a device equipped in the Philips TUV 36W/G36 T8 lamps, 253.7 nm) every 100 s (100–800 s) in doses from 2,32 to 18,56 kJ m^{-2}. The results of experiments were subjected to the statistical analysis. The theoretical time needed for microbial inactivation was calculated based on regression lines.

Results

The results of the study comparing the effect of nonthermal plasma and UV-C radiation on selected bacteria were presented both in form of graphs illustrating the number of microorganisms at different times of exposure to the factor, and as regression line equations in tables. The study indicated a varied sensitivity of bacteria to the action of both physical factors. Figure 1 shows that the number of *E. coli* was gradually decreased along with the time of action of nonthermal plasma. After 800 s the number of these bacteria was reduced by 6 log. UV-C radiation was characterized by a much lower effectiveness, as after the maximal time of exposure it reduced the number of the rods only by 1 log. From regression line equations (Table 1) it follows that the total survival time of *E. coli* in the environment of UV-C was almost five times longer than in plasma.

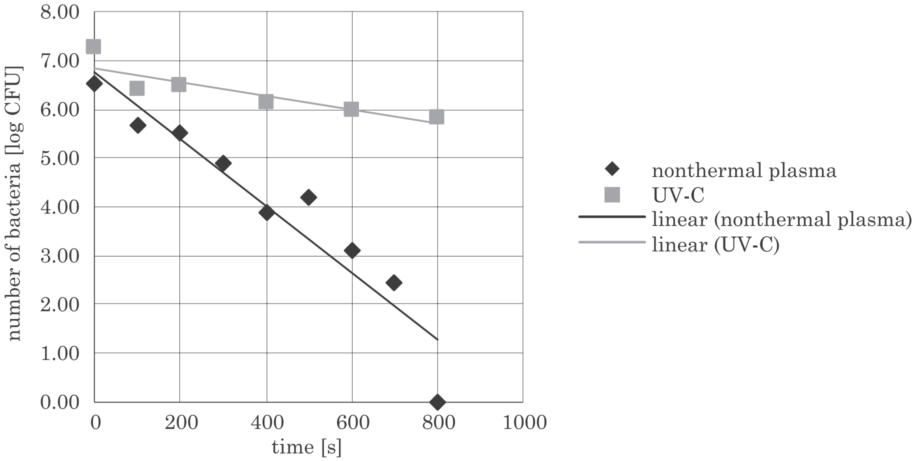

Fig. 1. Changes in the number of *Escherichia coli* as a result of action of nonthermal plasma and UV-C radiation

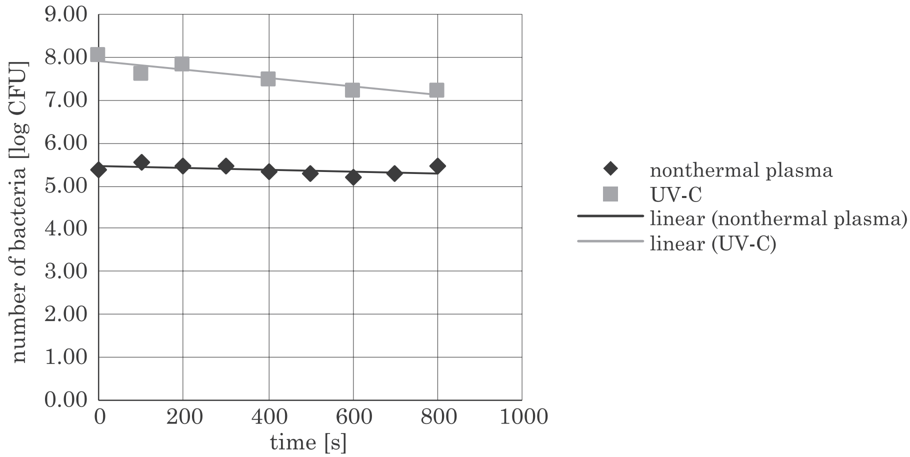

Fig. 2. Changes in the number of *Salmonella* Senftenberg W775 as a result of action of nonthermal plasma and UV-C radiation

The number of rods of *Salmonella* Senftenberg W775 did not change substantially either under the influence of plasma or UV-C and after 800 s it was comparable with the control sample (Figure 2). Regression equations indicate that UV-C radiation was more effective in their elimination. A decrease in the number of bacteria was more than two times faster than in the environment of plasma. This affected the total survival time of the cells, which was longer also after the use of UV-C (Table 1).

Table 1

Regression line equations describing the inactivation rate of bacteria under the influence of nonthermal plasma and UV-C radiation

Factor	Regression equations	Total survival time [min]	Fall of bacteria number [log/min]
Escherichia coli			
Plasma	y = -0.0069x + 6.7704	16.4	0.4
UV-C	y = -0.0014x + 6.8589	81.7	0.08
Salmonella Senftenberg W775			
Plasma	y = -0.0002x + 5.4598	455.0	0.01
UV-C	y = -0.001x + 7.913	132.0	0.06
Enterococcus faecalis			
Plasma	y = -0.0047x + 6.3881 y = -0,0002x + 6,1504	22.7	0.3
UV-C	y = -0.0002x + 6.1504	512.5	0.01

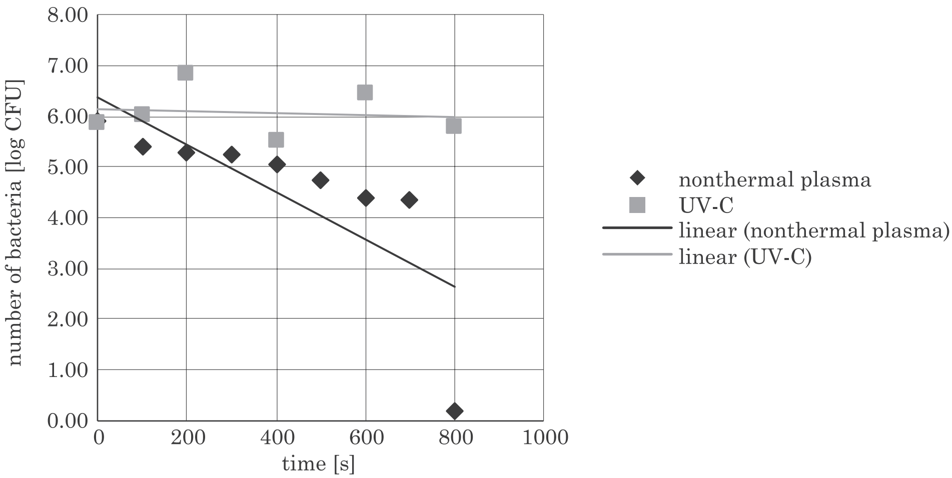

Fig. 3. Changes in the number of *Enterococcus faecalis* as a result of action of nonthermal plasma and UV-C radiation

Like *E. coli*, streptococci were effectively eliminated under the influence of nonthermal plasma. However, as long as until the 700th s their number fall much slower. Only during the last 100 s there was a reduction in the number of *E. faecalis* by 4 log as compared with the control sample. No bacteria inactivation was observed under the influence of UV-C radiation. As it follows from regression lines, streptococci were the most resistant to the action of UV-C radiation of all the bacteria. The total time of their survival was as long as 512 min, whereas in the environment of nonthermal plasma it was only slightly more than 22 min (Figure 3, Table 1).

Discussion

The possibility of using nonthermal plasma in the food industry is currently at the stage of experiments. Their results indicate wide possibilities for the future application of this technique for decontamination of surfaces of grains, seeds, meat or packages. This study tested the effect of low-pressure nonthermal plasma on the survival of faecal indicator bacteria *Escherichia coli* and *Enterococcus faecalis* as well as *Salmonella* Senftenberg W775.

DENG et al. (2007) tested the possibility of using nonthermal plasma (2 kHz) for inactivation of *Escherichia coli* on the surface of almonds. Already after 30 s they noted a decrease in the number of microorganisms by 5 logs. In the present study a similar decrease in the number of *E. coli* was obtained only after 700 s, which probably resulted from the use of a device with a much less power. A type of gas seems to have a large effect on the elimination rate of microorganisms in the plasma generator. PIGNATA et al. (2014) subjected *E. coli* adsorbed on membrane filter to the action of plasma in the environment of pure oxygen and argon for 15, 30, 60 and 180 s. Oxygen destroyed all bacteria after 60 s, and argon already after 30 s. In the present study, after 60 s of treating with plasma, the number of *E. coli* decreased only by more than 1 log, which indicates the fact that plasma generated in the atmospheric air is less effective in eliminating microorganisms than argon and pure oxygen.

ZHANG et al. (2013) studied the survival rate of *Salmonella* Typhimurium LT2, using spinach, lettuce, tomato and potato as carriers. Plasma was generated in conditions of lowered pressure with participation of oxygen. The device generated nonthermal plasma with the use of 13.56 MHz of radio frequency, pressure 34 Pa and power 150 W. After 600 s of the process, bacteria on spinach leaves were eliminated most efficiently, since a decrease in the number of cells by 3 logs was recorded. On the surface of lettuce, the number of microorganisms decreased by 2.75 log, whereas in tomato and potato, by 2.12 log. After 800 s, a very good effect of disinfection was obtained for spinach. In the present study, *Salmonella* Senftenberg W775 even after 800 s did not undergo too much reduction, as compared with the control sample. The used strain showed a high resistance to the action of nonthermal plasma. The result of the experiment could also be dependent both on a lower power and intensity of gas flow, different type of gas, and different types of bacterial carriers.

To compare the effectiveness of nonthermal plasma on microorganisms contaminating food, an experiment was made using the same carriers, but they were subjected to the action of more commonly used in food industry UV-C radiation. Relationship between the effectiveness of pathogenic microorganisms' inactivation and the UV wavelength was studied by YIN et al. (2015). They conducted experiments on cow milk, irradiating samples contaminated with *E. coli* O157:H7 with waves of different length and energy. Apart from the wavelength, also delivered energy had an impact on a reduction in the number of microorganisms. Both for 5 and 20 mJ cm^{-2} the highest reduction in the number of bacteria was observed at the wavelength 254 nm. BIRMPA et al. (2013) analysed the effect of ultraviolet radiation with the wavelength 254 nm on decontamination of the surface of fresh food products – Roman lettuce and strawberries contaminated with *Escherichia coli* and *Salmonella* Enteritidis. Samples were irradiated for 10, 20, 30, 45 and 60 min and a gradual reduction in the number of both bacteria species were recorded. After 13 min it was smaller by 0.4 log. In the case of strawberries, *Salmonella* sp. turned out to be more susceptible to the action of UV-C, after 13 min the number of microorganisms decreased by 0.8 log. In the present study after the same time of the action of UV-C the number of *Salmonella* and streptococci remained at the same level as the control sample, and the number of *E. coli* decreased by 1 log, that is over two times more than in the study by BIRMPA et al. (2013). Divergence in the study results could result from the use of different bacterial strains and carriers. The method of microorganisms' inactivation with UV-C radiation was also used by MUKHOPADHYAY et al. (2014). Their experiment aimed to assess the effectiveness of elimination of *Salmonella enterica* and *Escherichia coli* O157:H7 on the surface of fresh tomatoes. The device emitting radiation was equipped with lamps of a power 15 W, generating waves with a length of 254 nm. Tomatoes were irradiated by 100 s, in doses from 0.6 to 6 kJ m^{-2}. The number of both bacteria decreased along with an increase in dose of ultraviolet radiation. The rods of *Salmonella* sp. were characterized by a higher resistance.

The results of the present study also show a lower susceptibility of *Salmonella* to the action of ultraviolet radiation. The time of 800 s is insufficient to eliminate all microorganisms, including also the pathogens that occur in food products. To increase the effectiveness of decontamination, it would be necessary to increase the time and dose of irradiation.

It has been reported that many stressful conditions, including plasma treatment, or irradiation can lead bacteria to change into the VBNC state. The cells lose their ability to be cultivated, but still are alive and therefore may display pathogenicity. For this reason, the evaluation of decontamination processes of food might not be credible when only the classical cultivation methods are used (DOLEZALOWA, LUKES 2015, DING et al 2017, SAID et al 2010, ZHANG et al 2015). Further research should include some fluorescent techniques.

Conclusions

The plasma technology is more effective in bacteria reduction than ultraviolet radiation but nevertheless does not guarantee the full effectiveness of food decontamination. The treatment time and power of nonthermal plasma should be chosen so that the number of every indicator bacteria will be reduced. The real decontamination effectiveness of nonthermal plasma remains to be demonstrated by further research supplemented by VNBC analysis.

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